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WASHINGTON, D. C.



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## ERRATA AND AUTHORS' EMENDATIONS.

- Page 106, line 10, should read "western yellow pine (*P. ponderosa* Lawson, var. *scopulorum* Eng.), Douglas fir (*Pseudotsuga taxifolia* [Lambert] Britt.), lodgepole pine (*P. contorta*, Loud., = *P. murrayana* Ball.), and Engelmann spruce (*P. engelmannii* [Parry] Eng.), instead of "western yellow pine, Douglas fir, lodgepole pine, and Engelmann spruce."
- Page 106, line 13, should read "Lake States pines (White pine, *P. strobus* Linn., Norway or red pine, *P. resinosa* Sol., and Jack pine, *P. diversicata* Gordon) and" instead of "Lake States pines and."
- Page 105, footnote 5, third line from bottom, insert after the words "sour orange" the following "*Citrus aurantium* L. pomelo"
- Page 151, line 11, should read "bactericidal" instead of "bacteriacidal."
- Page 253, lines 15 to 16 from bottom, should read "*Andropogon scoparius*" instead of "*Andropogon scoparius*"
- Page 449, line 5, should read "they produced" instead of "they provided."
- Page 597, Table I, head "Fruits inoculated" should stand over columns 2, 3, 4, 5. Head "Fruits infected" should stand over columns 6 to 13.

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No. 1

## TEMPERATURE RELATIONS OF ELEVEN SPECIES OF RHIZOPUS<sup>1</sup>

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of Agriculture*

### INTRODUCTION

arter, Weimer, and Lauritzen (12)<sup>2</sup> have recently shown that the cal softrot of sweet potatoes may be produced by nine different ies of the genus *Rhizopus*. It has been demonstrated likewise that e fungi can produce a similar decay of a large number of fruits and ables when the host and parasite are brought together under f able conditions (11). The importance of temperature as one of the onditions essential for infection by these fungi was pointed out, it naving been found necessary in all cases to expose the hosts to tempera- tures within the range most suitable for the growth of the fungus in order to obtain infection. These fungi were placed roughly into high, ow, and intermediate temperature groups. More recent studies have een made<sup>3</sup> in which the temperature limits for growth of these fungi upon the sweet potato have been determined. The behavior of these fungi upon the living host is of special interest, since it is under these conditions that they become of economic importance. However, in studies of this nature two living organisms are involved, each of which may respond quite differently to various conditions of the environment. At high temperatures the physiological activities of the host are ac- celerated, while the reverse is true at low temperatures. The growth of the fungus will likewise be stimulated or retarded, depending upon he temperature employed, consequently any living host as a medium

testing the response of the species of *Rhizopus* to temperature would ot be uniform at different temperatures and would therefore be unsatis- factory. It was the object of these investigations to determine the influence of temperature on the development of 11 species of *Rhizopus* when grown upon an artificial culture medium of uniform composition.

The studies, the results of which are presented below, include the effect of temperature first on the germination of the spores, second on the growth of the mycelium, and third on the fruiting of the fungi.

### SPECIES STUDIED

The species of *Rhizopus* studied were the same as those used in former investigations: Namely, *nigricans* Ehrnb., *reflexus* Bainier, *chinensis* Saito,

<sup>1</sup> Accepted for publication May 29, 1922.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 39-40.

<sup>3</sup> By J. I. Lauritzen, of the Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture. The results have not yet been published.



*tritici* Saito, *artocarpi* Racib., *delemar* (Boid.) Wehmer and Hanzawa, *maydis* Bruderl, *nodosus* Namysl, *oryzae* Went and Pr. Geerligs, *microsporus* v. Tieg., and *arrhizus* Fischer. As previously explained (12) all the original cultures except those of *nigricans* and *arrhizus* were obtained from Mr. E. D. Eddy. Each organism was pure-lined by isolation from a single spore. A comparison with the original descriptions of each species showed no appreciable disagreement.

## METHODS

In order to keep the stock cultures in a vigorous state of growth they were renewed about once each month by transferring to sweet potato agar in small Erlenmeyer flasks and incubated at a temperature of from 22° to 26° C. Cultures of from 5 to 10 days old were used in the experiments. Preliminary investigations showed that *Rhizopus* spores germinate more readily in some good nutrient medium such as sweet potato decoction than in water, hence the former medium made previously described (10) was used.

In order to have available a solution of uniform composition for all the spore germination tests, a large volume of sweet potato decoction was prepared and stored in small flasks stoppered with cotton and covered with several thicknesses of oiled paper to retard evaporation. Each flask contained about 120 cc. of the decoction, which was about the amount required for the study of the spore germination of each organism. By storing the solution in small flasks the necessity for resterilizing the stock solution each time some of it was removed and thereby possibly changing its chemical composition was avoided. The loss by evaporation was restored by bringing the solution up to its original volume by the addition of distilled water. A loop of a spore suspension made in this medium was placed upon a clean cover slip, which was then sealed with vaseline in an inverted position to a glass ring cemented to a slide with a mixture of beeswax and vaseline. In the bottom of a cell so constructed was placed a small quantity of the same solution. The cells used were comparatively large (13 mm. high by 16 mm. in diameter) and provided sufficient air for the germinating spores.

In view of the fact that some investigators have found the hanging-drop method of studying spore germination unsatisfactory, the writers made several preliminary comparative tests of different methods. These showed that so far as these species of *Rhizopus* are concerned as reliable results could be obtained by the hanging-drop method as by any of the others, as, for example, in small volumes of solution in test tubes. Furthermore, since repeated observations must necessarily be made to determine accurately the time of germination the hanging-drop method lent itself more readily to manipulation than the test-tube method in which a fresh mount must be made for each observation. The hanging-drop cultures were placed in the incubators as soon as possible after they were prepared. The incubators were manufactured by Paul Altmann, were electrically controlled, and ranged in temperature from 1.5° to 50° C. Usually the temperatures in the individual chambers remained almost constant, varying for the most part less than a degree except in the lower ones, where there was sometimes a variation of 2° or 2.5° C. Pans of water were kept in the bottom of the chambers to keep the air moist. At least four and more often eight hanging drops were used at each temperature.

The rate of growth of these fungi at various temperatures was studied by growing them in Petri dishes containing 10 cc. of a 2 per cent Irish potato agar without additional sugar. A small drop of a suspension of spores in water was placed in the center of each plate with a 2-mm. platinum loop. The plates were placed in the incubators as soon as they were prepared, and the rate of growth was determined by measuring twice daily the diameter of the mycelial felt formed. Five Petri dishes were placed at each temperature. When the results were not entirely satisfactory the experiment was repeated.

The effect of temperature on sporangia formation was determined by growing the fungi at different temperatures in 100-cc. Erlenmeyer flasks on 30 cc. of 2 per cent Irish potato agar and observing the temperatures at which the sporangia were produced and the time required for their formation.

#### INFLUENCE OF TEMPERATURE ON SPORE GERMINATION

The effect of temperature upon spore germination has been studied by a number of investigators and by several different methods. The percentage of germination at different temperatures has been used as a measure of the influence of temperature upon spore germination by Melhus (14), Doran (4), Reed and Crabill (18), and others. Naturally the method used in any particular investigation must depend upon the type of data desired. Preliminary tests showed that practically 100 per cent of the spores of *Rhizopus* spp. germinated except at extreme temperatures, hence, the percentage of germination could be used as a measure of the effect of temperature only by taking into consideration the time factor. The number of spores which germinate and cause infection in the case of decay-producing fungi of this type is not of as much importance as it is in the case of organisms producing other types of diseases, such as leafspots. In the former case a host may be completely destroyed as a result of a single infection, while in the latter the total amount of damage done often depends upon the number of individual infections. In view of these facts the writers decided to use the time required for germination to begin as the measure of the influence of temperature upon this phenomenon. Anderson (2), Melhus (14), Ames (1), Rands (16), Ravaz and Verge (17), Shapovalov (19), and others have used this same criterion in their investigations. However, in no case is it made clear when these writers considered germination to have taken place. It is possible to use as a criterion the time when the maximum percentage of the spores have germinated or when the spores which germinate first have produced germ tubes. With *Rhizopus* a few spores in each drop produce a germ tube first, followed very soon by others, the number gradually increasing so that in a short time nearly 100 per cent of the spores have germinated. The period intervening between the time when the germ tubes appear on the spores which are the first to germinate and the time when all the spores have germinated varies with the temperature, being shortest at the optimum and gradually increasing as the upper and lower temperature limits are approached. As a criterion of germination the writers decided to use the time necessary for the spores which germinate first to produce a germ tube equal in length to the diameter of the spores. Using the figures thus obtained, curves were plotted which show the variation in time due to the difference in temperature. The time necessary for the germ tubes to reach some specific

number of microns in length might have been employed; but the measure selected was easier to determine, since the spore was always present with the germ tube for quick comparison, while considerable time would have been required to bring the spore in line with the micrometer scale for measurement. In these studies time was a very important factor, since germination often occurred in three or four incubators at nearly the same time, often not over from 5 to 15 minutes apart. Some care had to be exercised, especially at the less favorable temperatures, as often one or a very few spores would start to germinate considerably in advance of the remainder. However, by examining the hanging drop cultures frequently it was possible to determine with a fair degree of accuracy the point sought. Slides were examined near the incubators and were kept out usually less than a minute. Only at the higher temperatures was there any appreciable fluctuation as a result of opening the doors, and in such cases the normal temperature was quickly restored.

The results obtained from the study of the germination of the spores of 10 of the species is shown by the curves in figure 1. The germination of *Rhizopus maydis* spores was not studied for the reason that under the conditions under which the cultures were grown spores were not produced in sufficient quantity to carry out the experiment.

In the curves in figure 1 the time in hours necessary for the germ tubes to reach the length of the diameter of the spores was plotted on the abscissa, while the temperature in degrees centigrade was plotted on the ordinate. The maximum temperature for germination as indicated by the table is that temperature at which no germination took place. The temperature at which germination will just take place is very difficult to determine, since at 1° or 2° C below the maximum it frequently starts and then stops before the germ tube reaches the diameter of the spore. For example, *Rhizopus artocarp*i spores germinated readily at 32.3° while at 33.4° only about one-half of 1 per cent of the spores sent out germ tubes, which finally reached a length equal to twice the diameter of the spore and then stopped. At 34.5° the spores became somewhat swollen, which is a condition always preceding the extrusion of the germ tube. After 48 hours the spores had been killed at the two higher temperatures, as indicated by the fact that they failed to germinate when placed at a temperature favorable for germination. Similar results were obtained with other species. No attempt was therefore made to establish a definite maximum, but it may be said that in general it is somewhere from 1° to 2° lower than the temperature plotted as being the inhibiting temperature. In every case where there was no germination within 24 to 48 hours the spores were found to have been killed. The temperatures just above the maximum which inhibited germination of the spores of the different species are as follows: *artocarp*i, 34.5°, *tritici*, *delemar*, *oryzae*, and *arrhizus*, 45.5°; *chinensis*, 52°; *reflexus*, 38°; *nigricans* and *microsporus*, 34°. The lower temperature limits for germination for only two of the species, *nigricans* and *microsporus*, are shown by the curves, since in all other cases the time for germination at the minimum temperature was more than 100 hours. The spores of *chinensis*, *oryzae*, and *delemar* had not germinated during 30 days' exposure at 8.5°, 7°, and 7°, but germination did take place in that length of time at temperatures of 10°, 9°, and 8.7°, respectively. On the other hand, the spores of the remaining species, *reflexus*, *arrhizus*, *tritici*, *nodosus*, and *artocarp*i, germinated at 1.5°, the lowest temperature tried, in 5, 15, 22, 14, and 6 days, respectively. The percentage of spore germination at

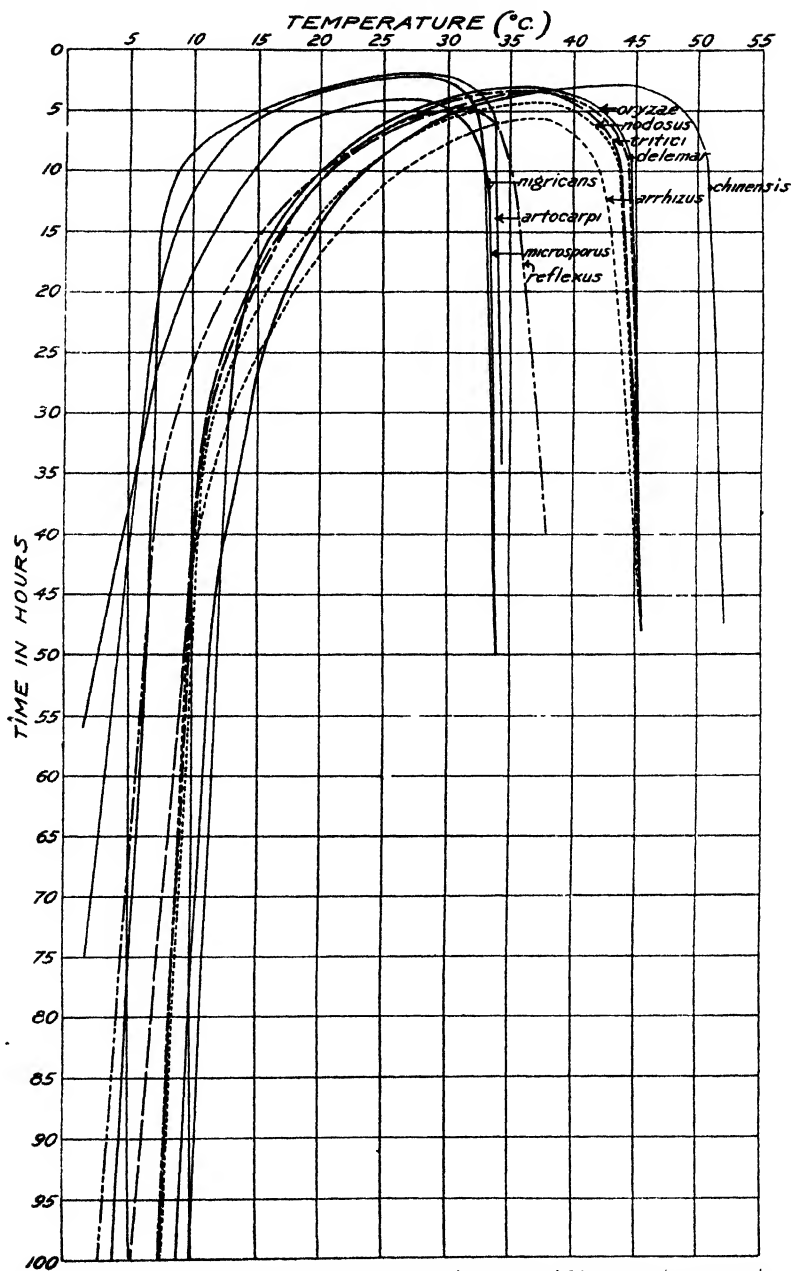


FIG. 1.—Curves showing the time necessary for the spores of 11 species of *Rhizopus* to form germ tubes equal in length to the diameter of the spore at different temperatures

this temperature was often small, and only in the case of *reflexus* did any appreciable growth take place within the time limit of these experiments (30 days).

The optimum temperatures as obtained by this method are as follows: *artocarp*, 26° to 29° C.; *nigricans* and *microsporus*, 26° to 28°; *tritici*, *delemar*, *nodosus*, *oryzae*, and *arrhizus*, 36° to 38°; *reflexus* 30° to 32°; *chinensis*, 43° to 45° C. Any attempt to determine a closer optimum seemed impracticable, since the rate of germination at these temperatures is so nearly the same. For the convenience of the reader and for the greater ease of comparing the effect of temperature on germination, growth, and fruiting these data are set forth in Table I and will be referred to later.

The curves (fig. 1) show that the species studied fall into three groups according to their response to temperature. *Chinensis* stands out conspicuously as a species with a high optimum, although there is a considerable range through which good growth will take place. In fact, this species has a wider temperature range, about 30° C., through which the spores germinate readily, than any of the others studied, which indicates that it is less sensitive to heat. *Artocarp* probably stands at the other extreme, although its spores germinate well within a range of 20° (10° to 30°). The drop on both sides of the curve is very abrupt. Owing to its sensitivity to heat considerable difficulty was at first experienced in keeping this fungus alive. This species was the most erratic in its behavior, requiring more study to determine its temperature relations than any of the other species.

Along with *artocarp* as species with comparatively low optimums and maximums may be placed *reflexus*, *nigricans*, and *microsporus*. The results seem to indicate that the two latter species have the lowest minimum, since their spores germinated much more quickly at the lowest temperature tried.

The remaining five species are very similar in regard to their maximum and optimum temperatures, differing only in the time necessary for germination, which varies at the optimum from 3 hours (*oryzae*) to 5½ hours (*arrhizus*). As pointed out above, however, these species also differ in their lower temperature limits in that the spores of *oryzae* and *delemar* failed to germinate at 7° C. while those of the other three germinated at 15°.

The temperature relations of several species of *Rhizopus* have been studied by Hanzawa (9) and Lendner (13, p. 111-127). The former separated *nigricans* from the other species studied by him by the fact that it was the only one which did not grow at 37° C., and the latter separated it from *oryzae* because it did not grow on potato at 39°. Hanzawa states that *nigricans* would not grow at blood temperature (35° to 37°). He also found that *chinensis* spores did not germinate at 6° and that those of *delemar* would not germinate below 12° or above 42°. Hagem (8), working with *Mucor* (*Rhizopus*) *nodosus* (Namyslowski), found that its spores did not germinate at 43° to 44° C. Ames (1) found that the spores of *R. nigricans* failed to germinate at 1° or at 42° but germinated at 3° to 4° and 41°, the optimum being from 38° to 41°, where germination took place in 5½ hours. Stevens and Wilcox (20) found that this fungus could mature a few sporangia on ripe strawberries at 36° to 37°. The difference in temperature limits given by different investigators for this fungus is difficult to understand. This disagreement in results might be due to several causes, such as the use of different strains, to

incorrect identification or to mixed cultures, to the influence of the culture media, etc. A comparative study of different strains of *nigricans* which the writers have under way may throw some light upon this subject. The variation in temperature between different parts of an artificially heated incubator is a factor which can not be overlooked. The upper strata of air in an incubator whose temperature is measured by means of a thermometer inserted through an opening at the top has been found to be in some cases from 1 to several degrees warmer than that at the center or bottom. In chambers heated by a water jacket the writers have recorded a difference of 5° between the temperature of the air at the top and bottom of a chamber, a distance of only 16 inches. In the present investigations the thermometers were compared with a standardized thermometer and then the bulbs were lowered to the immediate vicinity of the cultures in order to obviate as nearly as possible this source of error.

Dunn (5) found that the plus and minus strains of *nigricans* with which she worked differed somewhat in their temperature relations. The minus strain seemed to be more vigorous, since it had a slightly higher optimum than the plus and grew at both a slightly higher and a lower temperature. The optimum for growth by the minus strain lay between 25° and 28° C, which agrees closely with that obtained by the writers—approximately 25°. The maximum temperature for the growth of the minus strain was about 31°, which also agrees closely with that obtained for the strain studied by the writers. Dunn found a considerable difference in the optimums for different strains of *Rhizopus* from strawberries. The optimum temperature for one strain was about 36°, while that for other strains was approximately 27° or 28°. However, she expresses doubt as to whether she was working with strains or species, and since several species of *Rhizopus* are capable of decaying strawberries (11) she may have worked with a species which thrived best at a higher temperature rather than with a strain.

#### INFLUENCE OF TEMPERATURE ON GROWTH

Having determined the effect of temperature on germination, its influence on the continued growth of the germ tubes and resulting mycelium was next studied. This was done by measuring the daily increment of growth on agar in Petri dishes. The results are shown graphically in figures 2 to 12, in which the base line shows the temperature in degrees centigrade and the perpendicular shows the diameter of the mycelial disks at stated intervals of time.

An examination of these figures shows that they possess some common characteristics. Each series, for example, originates at scattered points to the left (the lowest temperatures tried at which growth did not take place within the given time), then rises in the direction of the optimum, and finally falls to a point at the right (the temperature at which no growth took place). From these figures it is evident that some growth took place at the maximum temperatures during the first 24 hours, while at the minimum temperatures growth did not begin until sometime later. The optimum temperature in all cases remains the same within the time shown in these graphs.

The lowest temperature at which growth of *maydis* and *arrhizus* was observed was 7.4° C. after 7 and 12 days, respectively. Hanzawa (9) found that *arrhizus* could make some growth at 6°. The writers did not

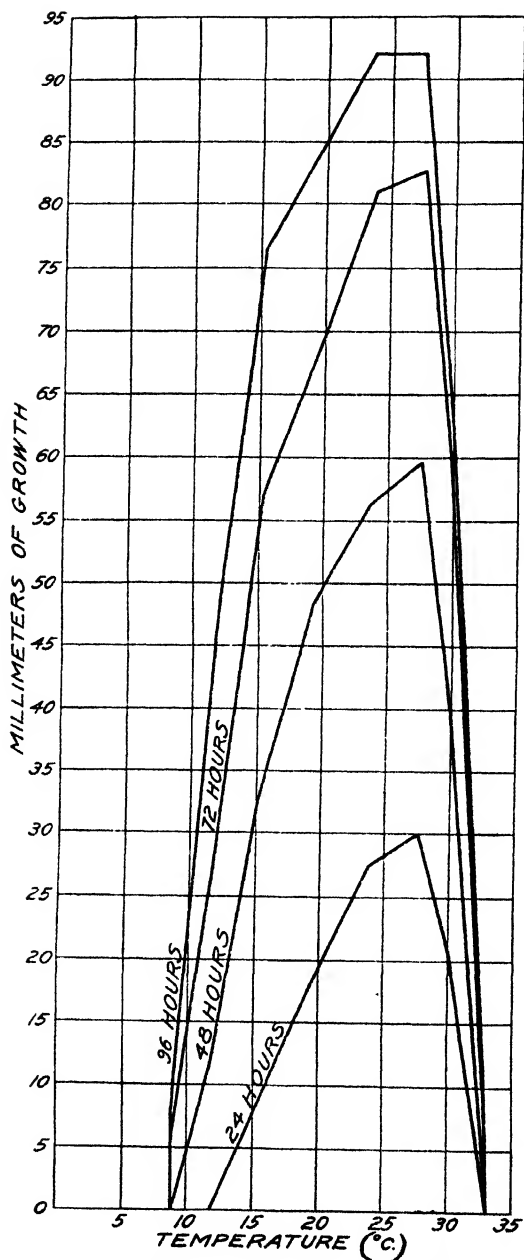


FIG. 2.—Graph showing the rate of growth of *Rhizopus arlocarpus* at different temperatures.

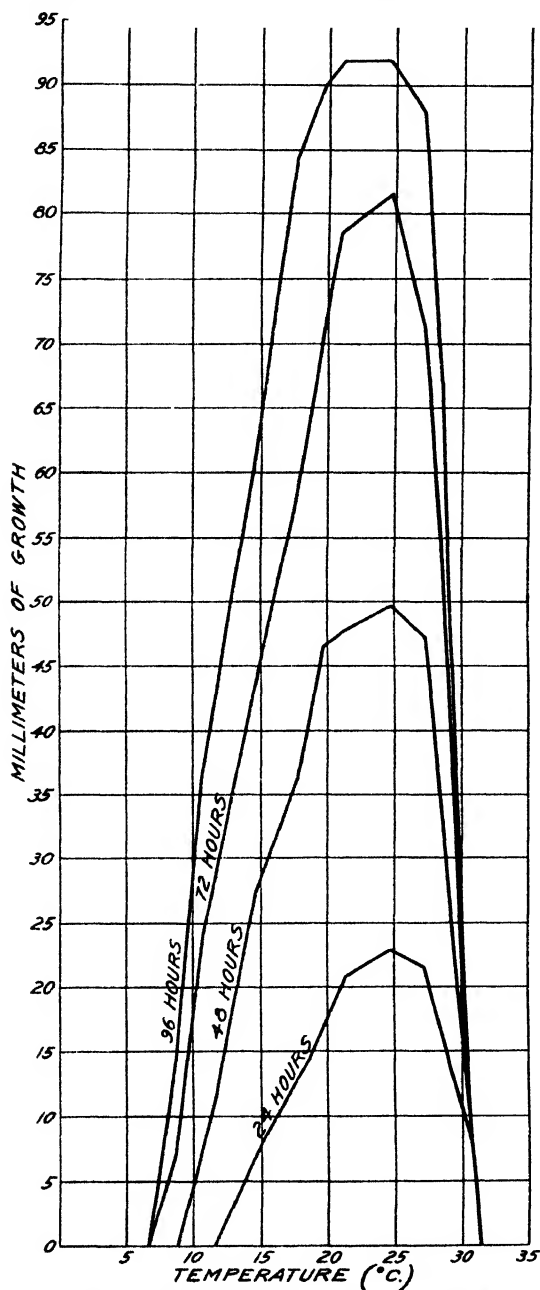


FIG. 3.—Graph showing the rate of growth of *Rhizopus nigricans* at different temperatures



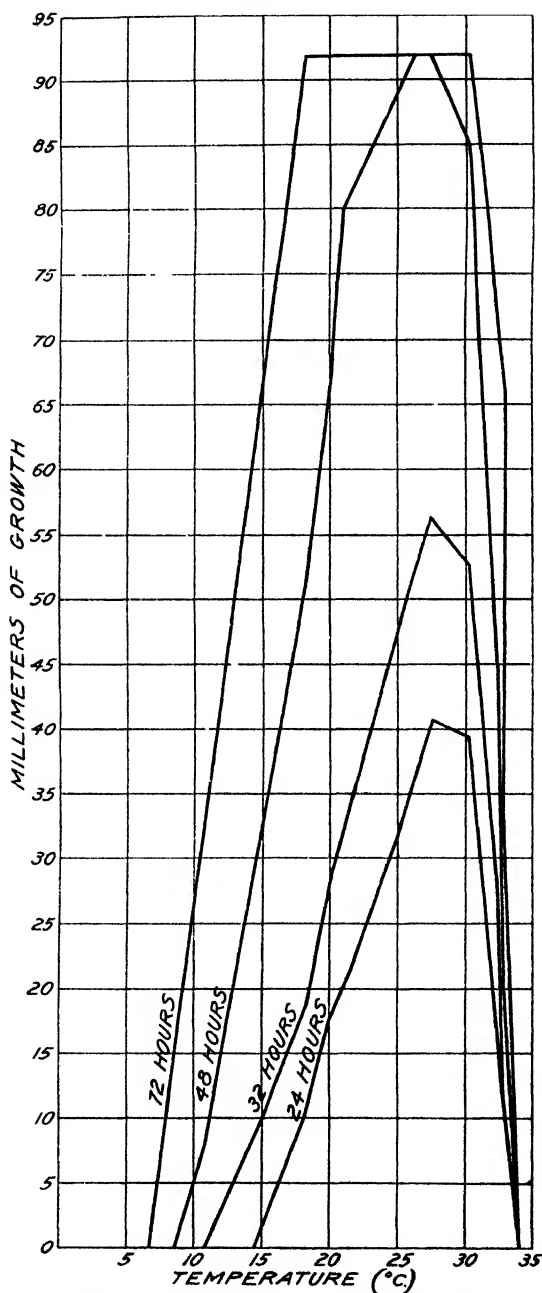


FIG. 4.—Graph showing the rate of growth of *Rhizopus reflexus* at different temperatures.

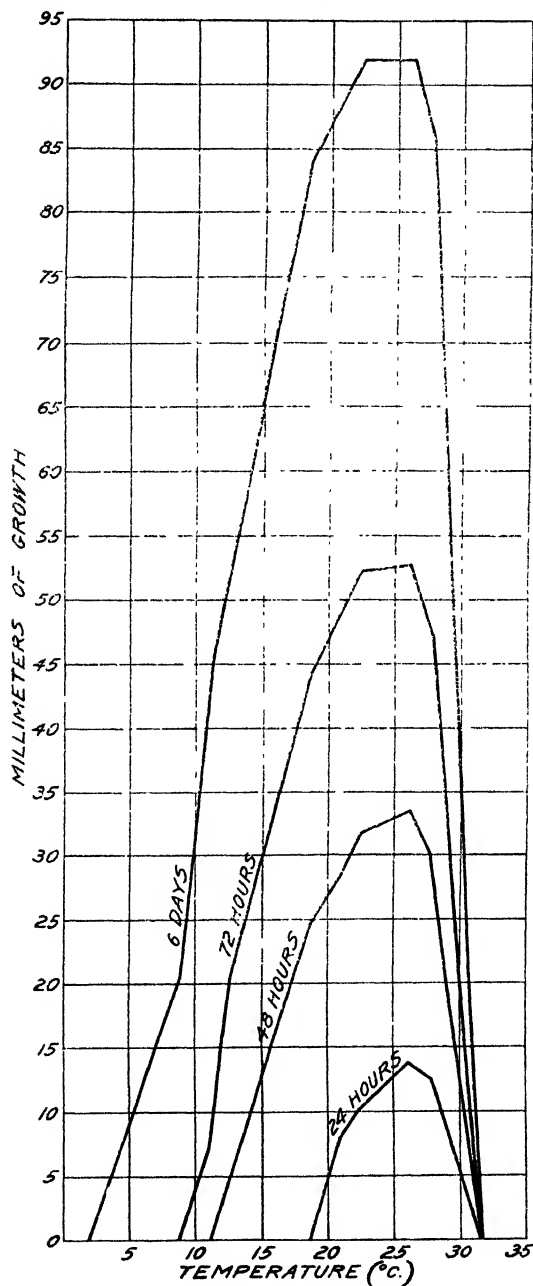
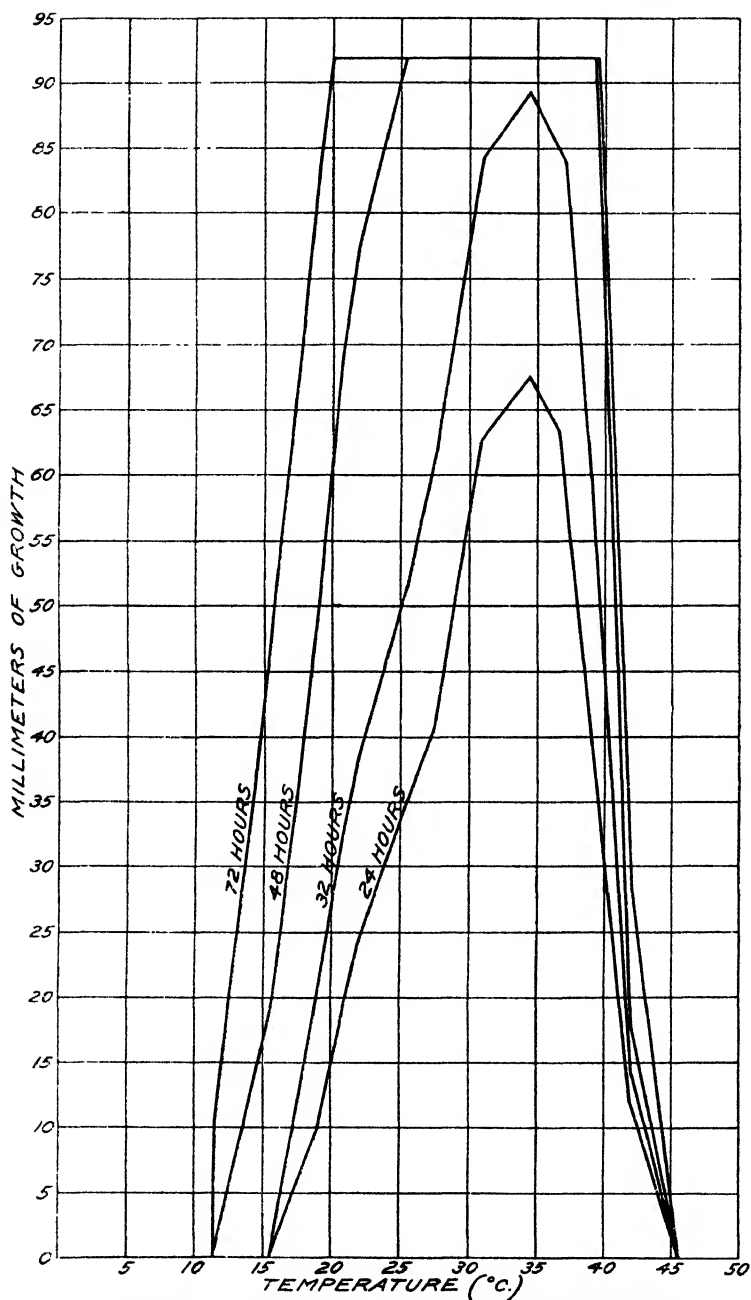


FIG. 5.—Graph showing the rate of growth of *Rhizopus microsporus* at different temperatures.

FIG. 6 --Graph showing the rate of growth of *Rhizopus tritici* at different temperatures.

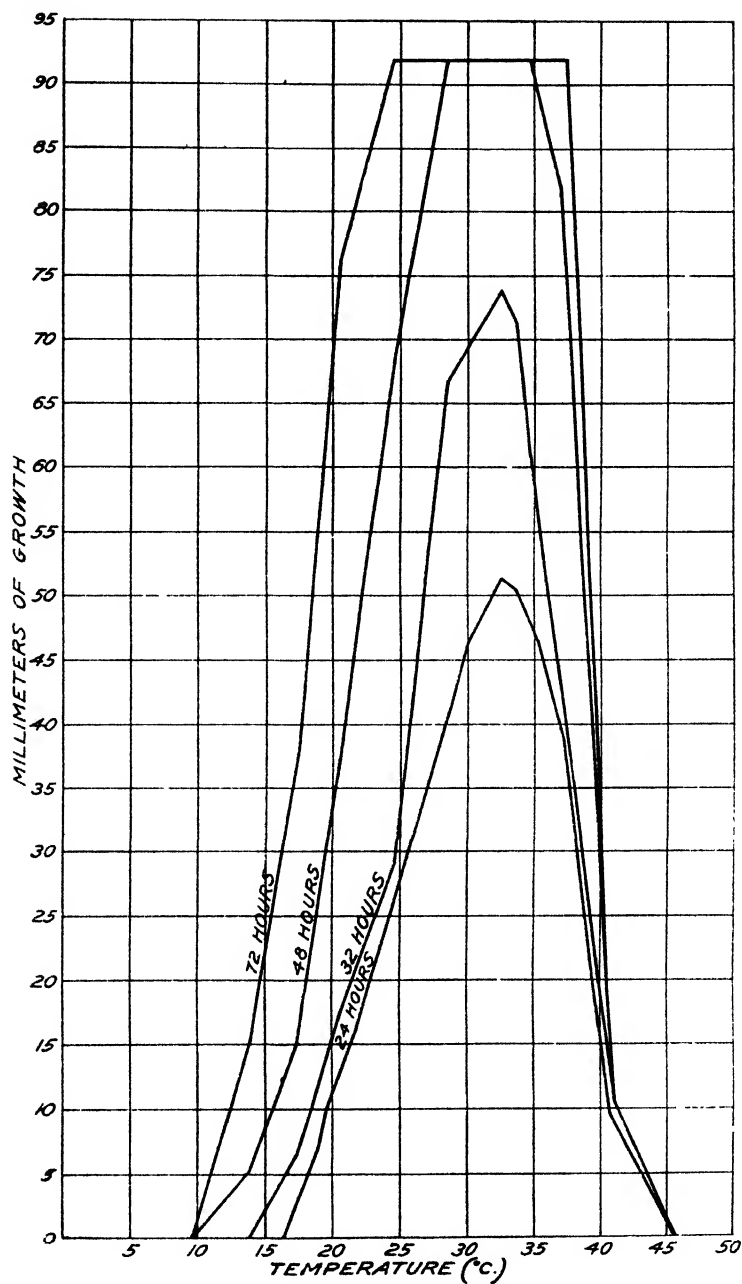


FIG. 7 —Graph showing the rate of growth of *Rhizopus delmar* at different temperatures

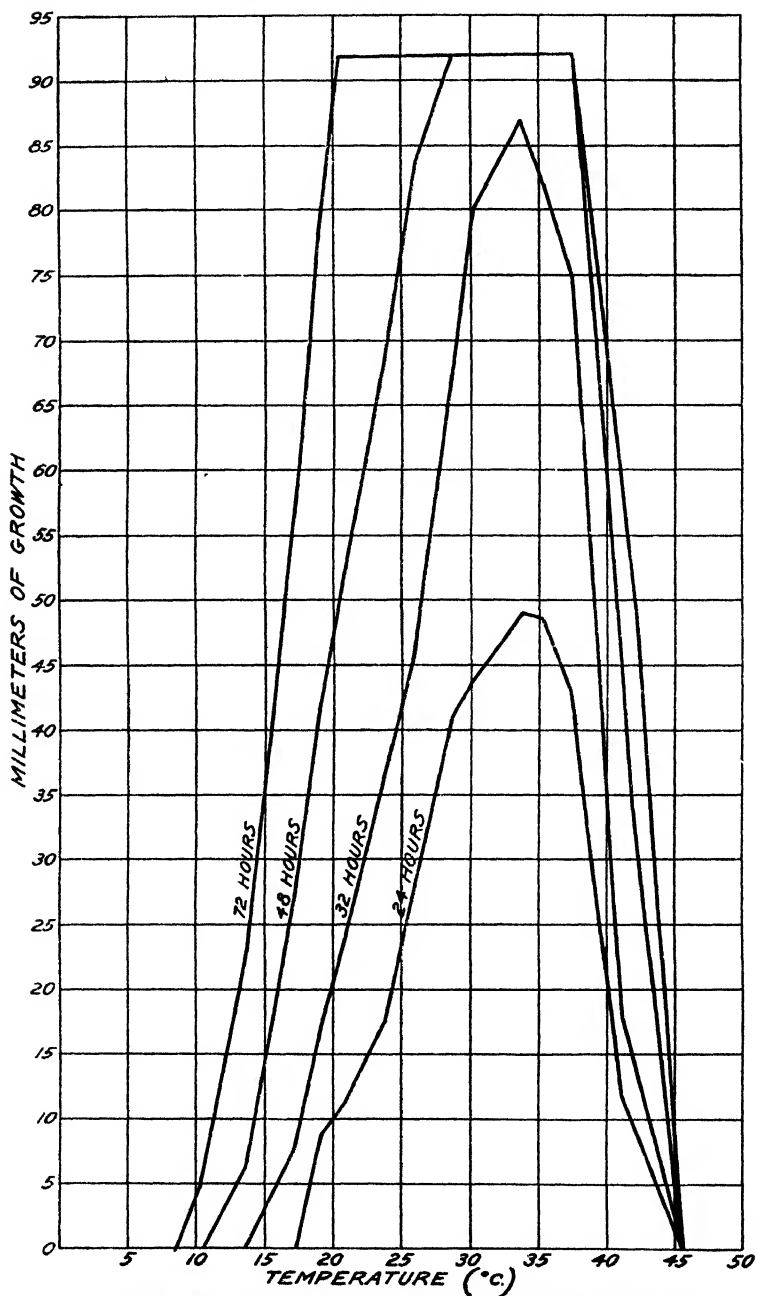


FIG. 8.—Graph showing the rate of growth of *Rhizopus nodosus* at different temperatures

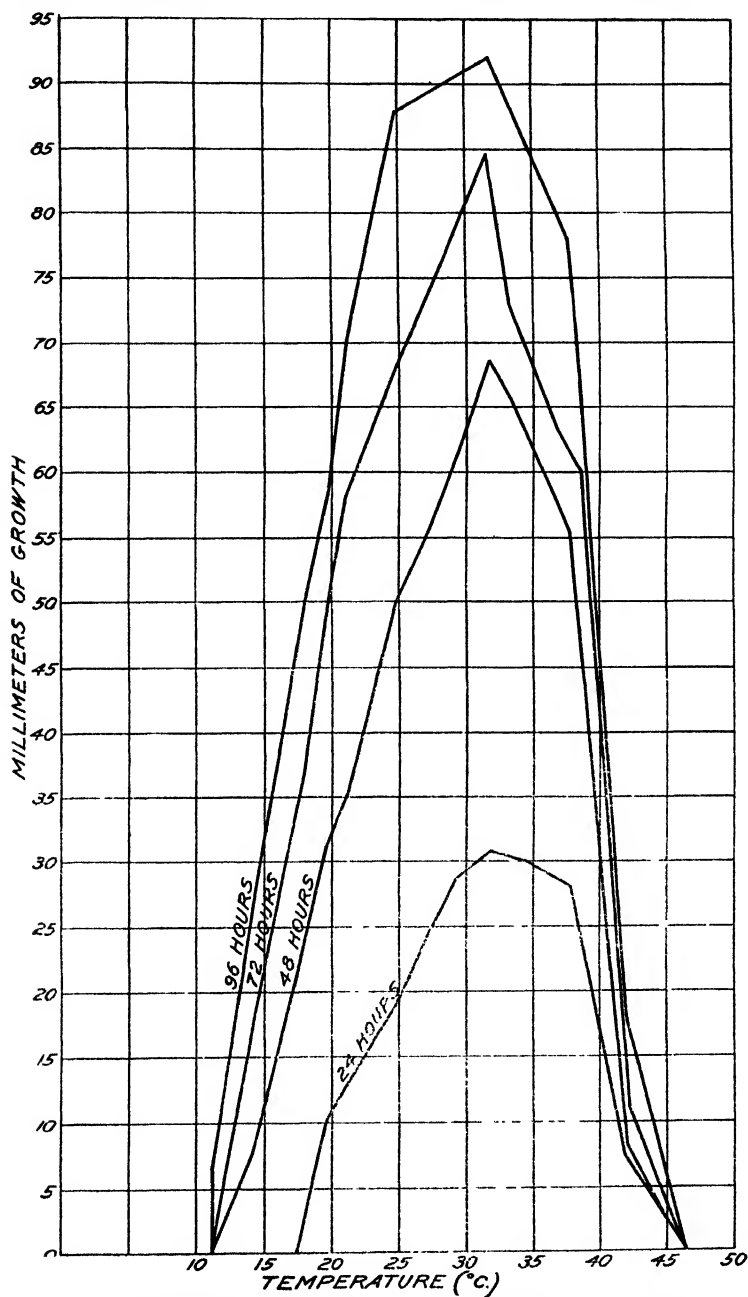


FIG. 9.—Graph showing the rate of growth of *Rhizopus oryzae* at different temperatures.

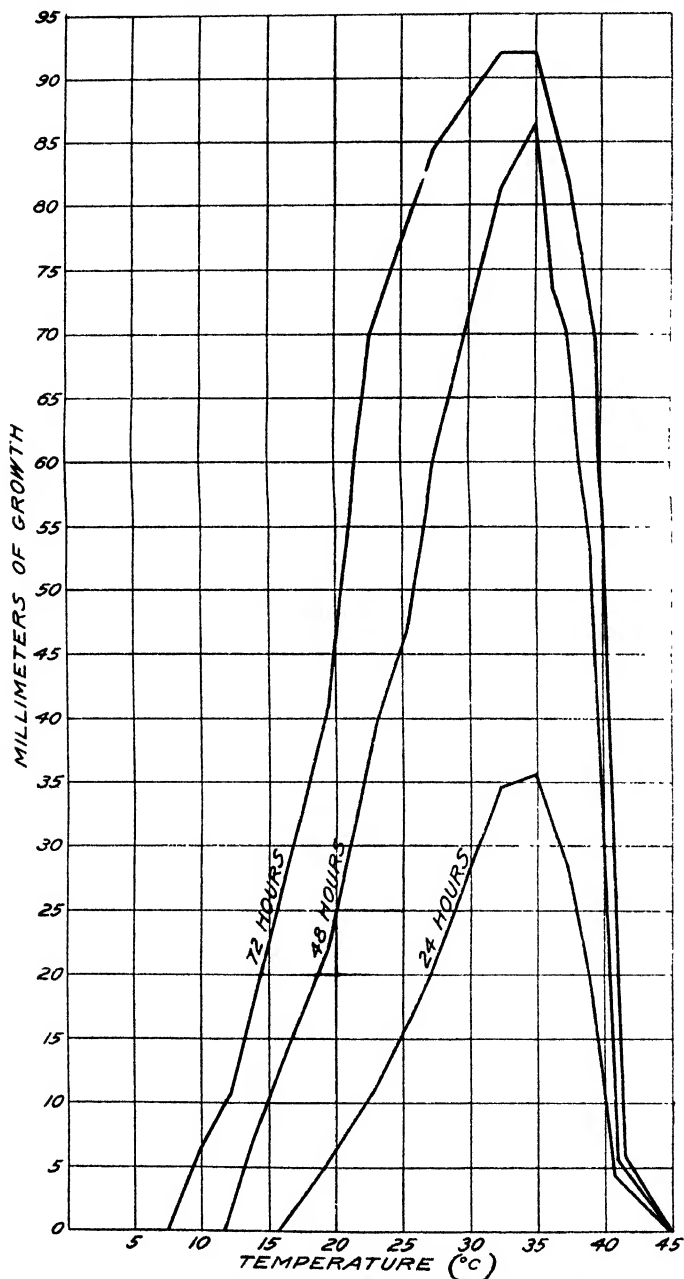


FIG. 10.—Graph showing the rate of growth of *Rhizopus arrhizus* at different temperatures.

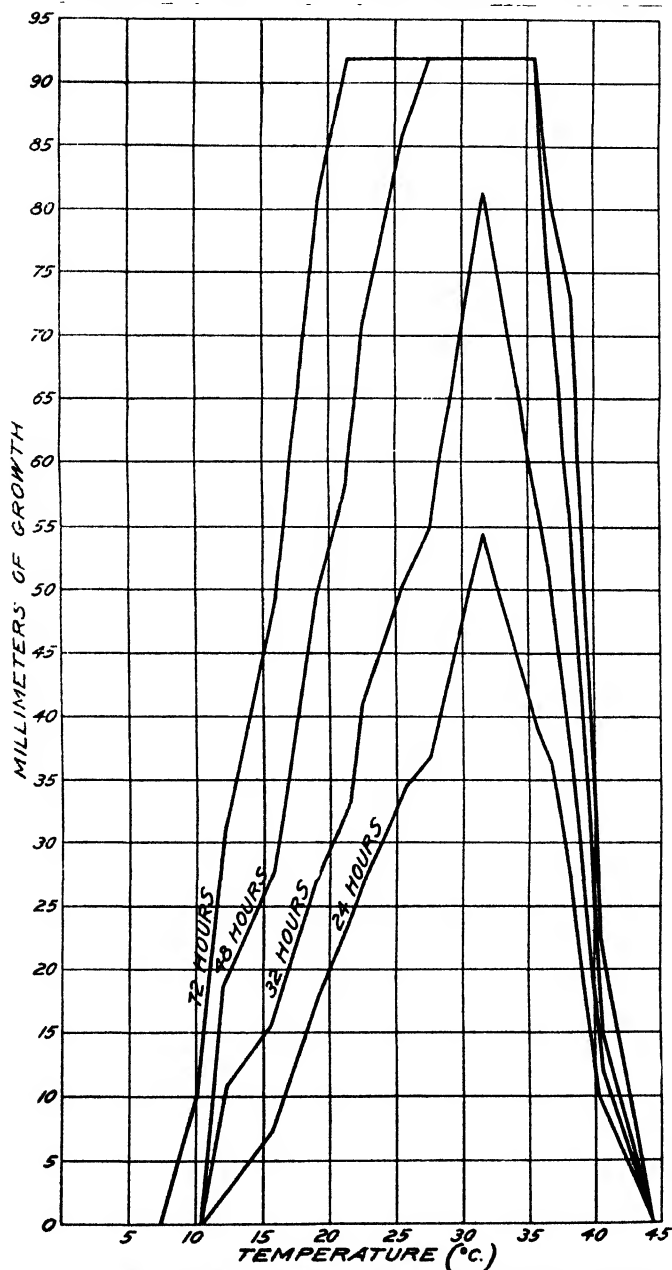


FIG. 11.—Graph showing the rate of growth of *Rhizopus maydis* at different temperatures :0615—23—2



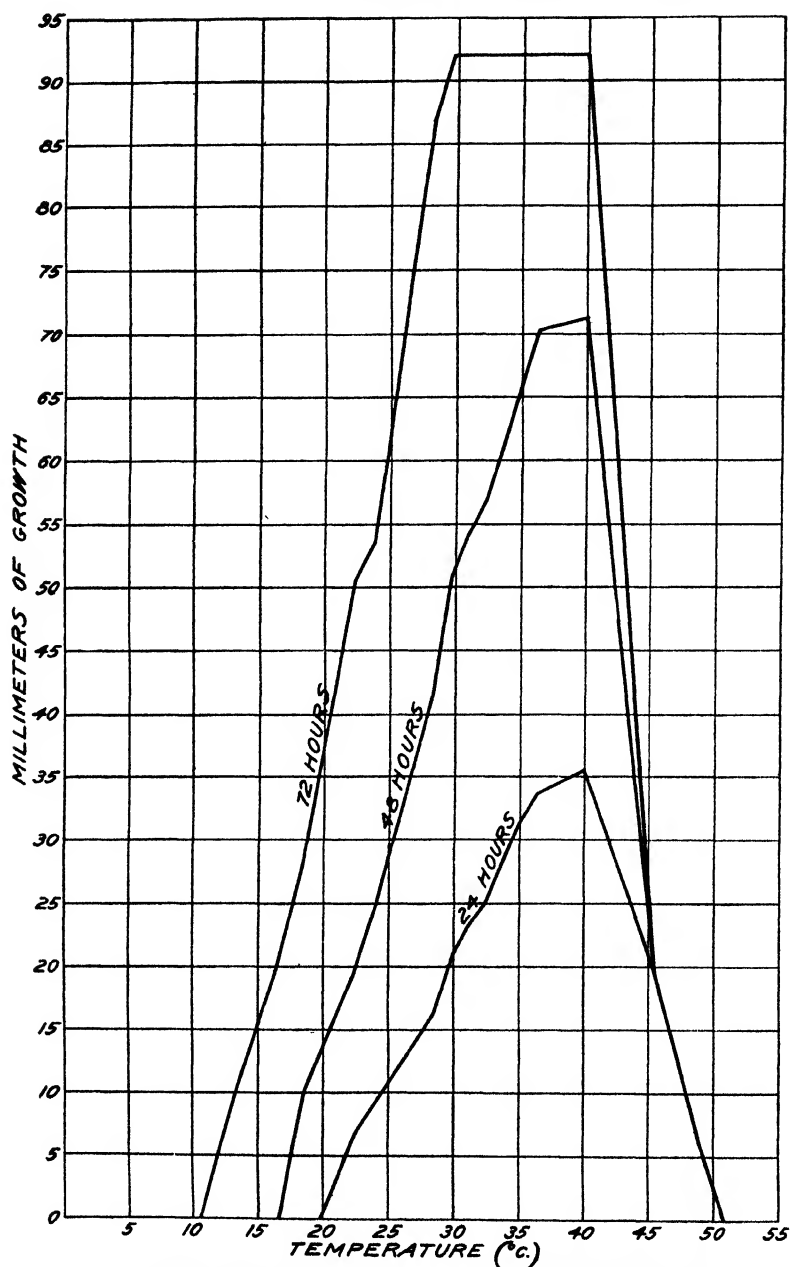


FIG. 12.—Graph showing the rate of growth of *Rhizopus chinensis* at different temperatures

try a temperature between  $7.4^{\circ}$  and  $1.5^{\circ}$ , but it is quite probable that growth will take place below  $7.4^{\circ}$  if sufficient time is allowed, since the spores germinated at  $1.5^{\circ}$ . Growth by *tritici*, *nigricans*, and *nodosus* at  $6.5^{\circ}$  began after 12, 8, and 10 days, respectively. These fungi also made some growth at  $6^{\circ}$  to  $8^{\circ}$  in Hanzawa's experiments. *Microsporus* and *reflexus* are the lowest temperature forms so far as growth is concerned. They began to make an appreciable growth at  $1.5^{\circ}$ , the lowest temperature tried, after 10 days. *Artocarpus*, a low-temperature form as indicated by its optimum, made some growth at  $9^{\circ}$  but none at  $7^{\circ}$ , while *chinensis*, which has a high optimum, made some growth at  $10.4^{\circ}$  but none at  $7.5^{\circ}$ ; in other words, growth was inhibited at practically as high a temperature in the low- as in the high-temperature form. At  $11^{\circ}$  and  $9.8^{\circ}$  *oryzae* and *delemar*, two forms very closely related both morphologically and physiologically, made some growth in 4 and 16 days, respectively. However, at  $7.5^{\circ}$  growth was entirely inhibited. *Oryzae* was not subjected to any temperature between  $11^{\circ}$  and  $7.5^{\circ}$ , so it is possible that some growth might have taken place at a temperature somewhat below  $11^{\circ}$ . Hanzawa found that this species made some growth at  $6^{\circ}$ , while the upper and lower limits for *delemar* were found to be  $12^{\circ}$  and  $42^{\circ}$ , respectively. It may be said, then, that the minimum temperature for growth varies with the time for the first 5 to 15 days, depending upon the species. After that time the true minimum—that is, the lowest temperature at which growth will take place regardless of the time—is reached. Thus growth decreases as the temperature is lowered until a point is reached where it is entirely inhibited. However, cultures which failed to grow after 30 days at the inhibitive temperature or lower develop rapidly when transferred to a more favorable temperature, showing that the protoplasm of the spore has suffered no harmful effects. These results are in general accord with those of other workers with other organisms.

It was pointed out above that there was visible growth at the maximum temperature by the end of the first 24 hours in each species. It would appear from the graphs that the maximum temperature in all cases is fixed—that is, that it does not change during the course of the experiments as does the minimum. However, this is not the case. *Delemar*, for example, made a growth about 9 mm. in diameter at  $41^{\circ}$  C. in the first 24 hours, which reached 10.5 mm. during the following 24-hour period and then ceased altogether, the mycelium failing to grow when placed at a favorable temperature. In this case the maximum for the first 48 hours was about  $41^{\circ}$ , after which even at  $38^{\circ}$  the growth was considerably retarded although not inhibited within the time limit of this experiment. Shifting of the maximum was noted with the other species. No doubt if temperatures sufficiently close were tried over a considerable length of time and by a method sufficiently delicate to determine accurately very small increments of growth a so-called shifting of the maximum would be found in all cases. As the temperature rises above the optimum a retarding soon followed by an inhibiting effect is noted. A comparatively short exposure to the inhibiting temperatures results in the death of the organism, although it is possible that a temperature slightly below the maximum established might be found which would inhibit growth for a considerable length of time and yet would not kill the fungus. Fawcett (6) has found a similar shifting of the maximum. Shifts from  $36^{\circ}$  to  $31^{\circ}$ ,  $38^{\circ}$  to  $35^{\circ}$ ,  $46^{\circ}$  to  $35^{\circ}$  were noted in *Pythiopsis citrophthora* Smith and Smith, *Phytophthora terrestris* Sherbakoff, and *Diplodia natalensis* Evans, respectively. All of these fungi

showed a lowering of the apparent optimum of from  $3.5^{\circ}$  to  $6^{\circ}$ . *Phomopsis citri* Fawcett, however, did not show similar changes. No such change in the optimum for growth of the species of *Rhizopus* studied by the writers was noted. It is possible that if the fungi had been grown for longer periods of time some such shifting of the optimum would have

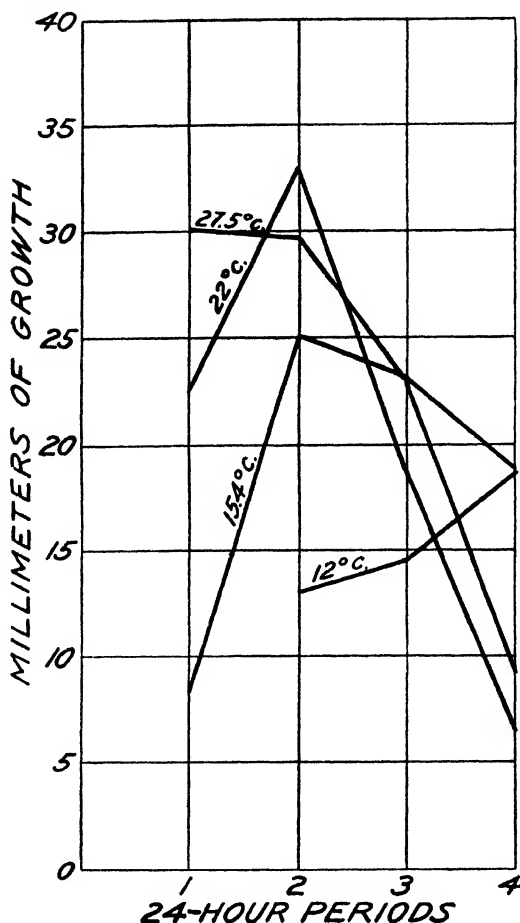


FIG. 1. —Graph showing the increase of growth of *Rhizopus arrhizus* for consecutive 24-hour periods.

become apparent. Hanzawa (9) found that the cardinal temperatures for some of these fungi were as follows: *Delema*, *nigricans*, *arrhizus*, and *nodosus* have maximums of  $42^{\circ}$ ,  $35^{\circ}$  to  $37^{\circ}$ ,  $42^{\circ}$ , and  $43^{\circ}$  to  $44^{\circ}$ , respectively; *oryzae*, *tritici*, and *chinensis* all made a good growth at  $38^{\circ}$  to  $42^{\circ}$ ; while the optimums for *delema*, *oryzae*, and *tritici* were  $30^{\circ}$ ,  $30^{\circ}$  to  $40^{\circ}$ , and  $30^{\circ}$  to  $35^{\circ}$ , respectively. Hagem (8), on the other hand, found that

*nodosus*, *nigricans*, and *arrhizus* had for their upper limits  $43^{\circ}$  to  $44^{\circ}$ ,  $33.5^{\circ}$ , and  $42^{\circ}$ , respectively; Lendner (13) gives  $30^{\circ}$  to  $35^{\circ}$  as the optimum for *tritici* and  $30^{\circ}$  to  $40^{\circ}$  as that for *chinensis*; while Bruderlem (3) states that *maydis* makes its optimum growth at  $39^{\circ}$ . This is considerably higher than that obtained by the writers. No explanation for this apparent difference can be given

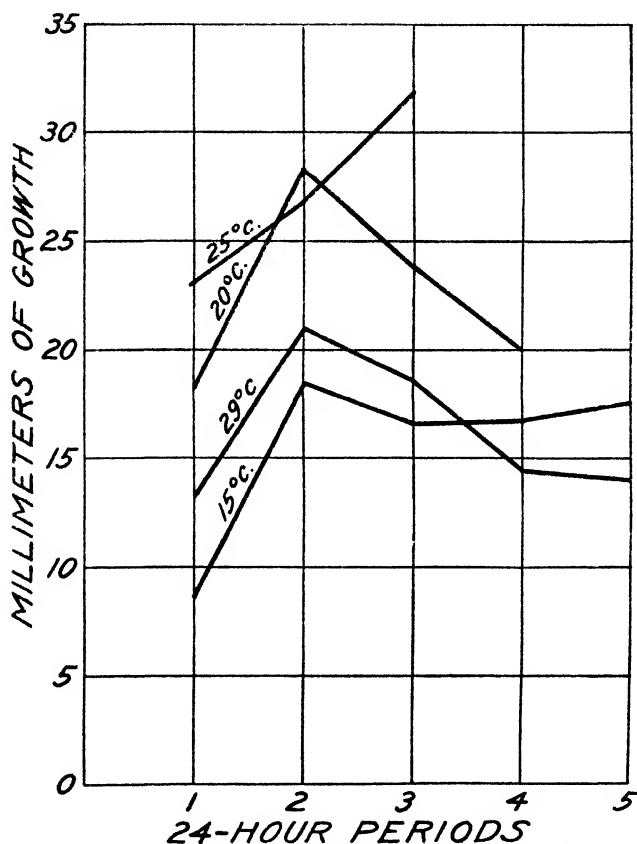


FIG. 14.—Graph showing the increase of growth of *Rhizopus nigricans* for consecutive 24-hour periods

Graphs (fig. 13 to 23) were constructed to show the variation in the growth rate at different temperatures for successive 24-hour periods. The ordinates represent diameter increase in millimeters while the abscissas represent successive 24-hour periods after exposure to a given temperature. An inspection of these graphs shows that for most of the fungi the maximum rate of growth was attained during the second 24-hour period, the exceptions being *delemar* and *chinensis*, which reached their maximums one day later. The data for *nodosus*, although not alto-

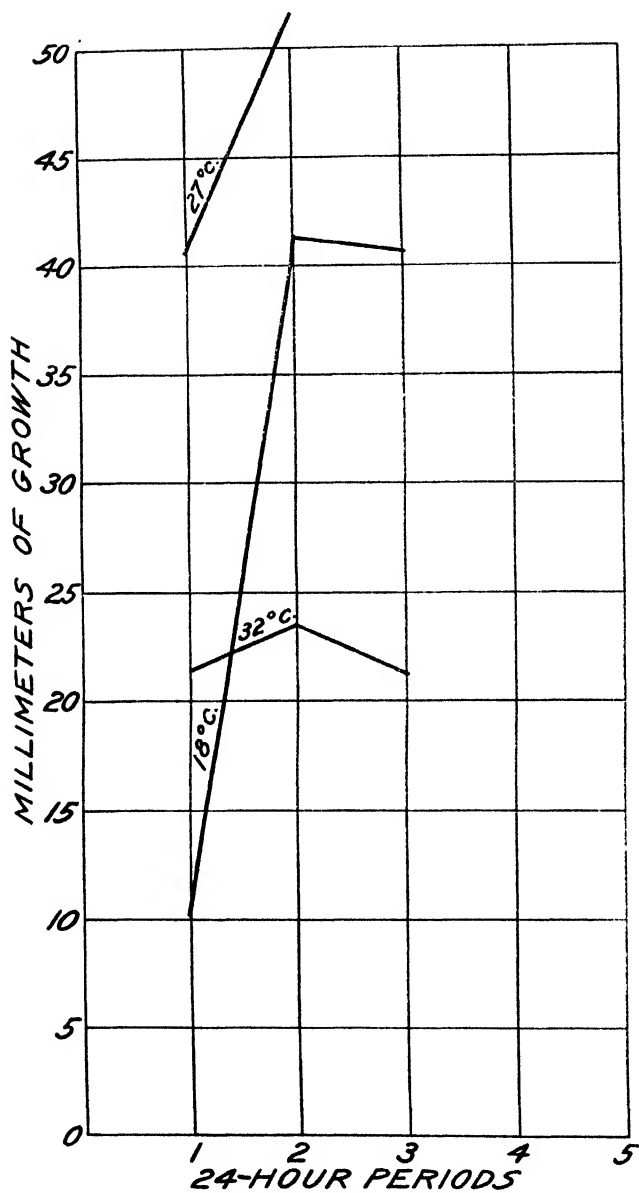


FIG. 15.—Graph showing the increase of growth of *Rhizobus reflexus* for consecutive 24-hour periods

gether consistent, seem to indicate that it makes its most rapid growth some time after the second day. The growth rate varied with the temperature in most cases. In *arrhizus*, *tritici*, *maydis*, and *artocarp*i the obvious discrepancies are at the low temperatures, where the curve continues to rise after the second day. Certain of the fungi—*oryzae*, *artocarp*i, *arrhizus*, and *nigricans*—show a rather rapid decline in the rate of growth after the maximum is attained, while others, such as *microsporus*, *delemar*, and *reflexus*, show a more gradual decline. In *chinensis*, so far as the data show, there was no reduction in the rate of growth up to the time the plates were covered.

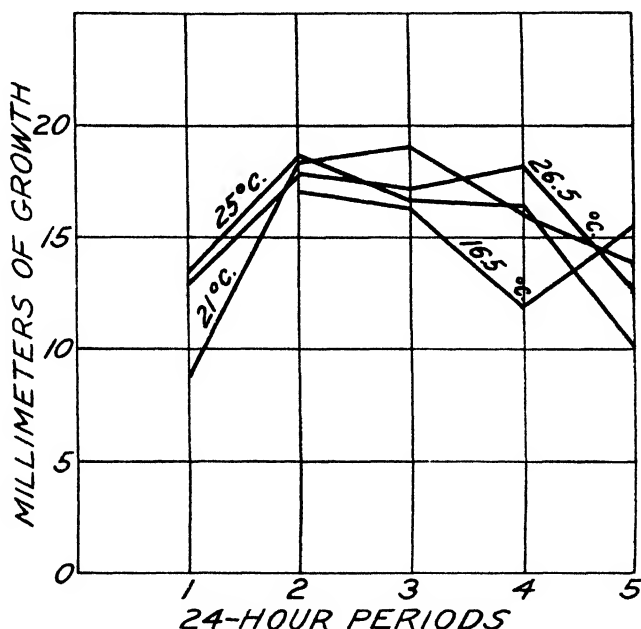


FIG. 10.—Graph showing the increase of growth of *Rhizopus microsporus* for consecutive 24-hour periods

Fawcett (6) found that the fungi investigated by him made their greatest increase in growth during the first two days at the lower temperatures and continued to increase at a more gradual rate thereafter. Over a small range of the higher temperatures the rate first increased and then remained more or less constant till the end of the experiment, while at the highest temperatures a continuous decrease was noted from the first observational time. In general these results agree with those of the writers. Only in *oryzae*, *artocarp*i, and *tritici* at the highest temperatures (38°, 27.5°, and 42° C., respectively) do the graphs show a decrease, beginning with the first 24 hours. Nevertheless the fact that the maximum shifts, as explained above, indicates that such a condition would have been found to exist had the proper temperatures—for example, temperatures very near the maximum—been used.

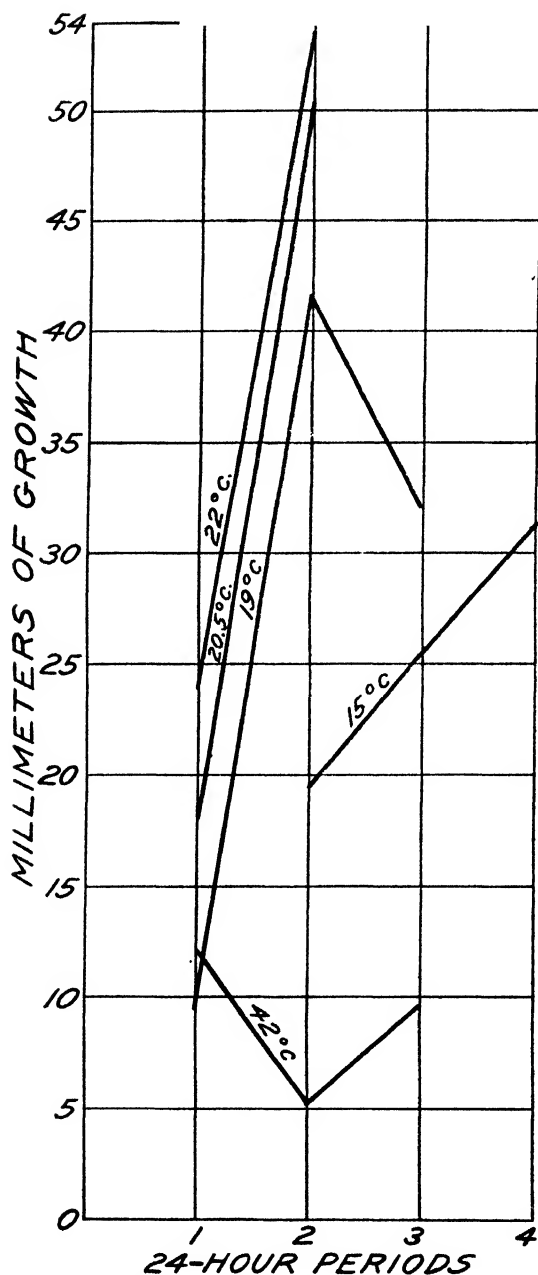


FIG. 17.—Graph showing the increase of growth of *Rhizopus tritici* for consecutive 24-hour periods.

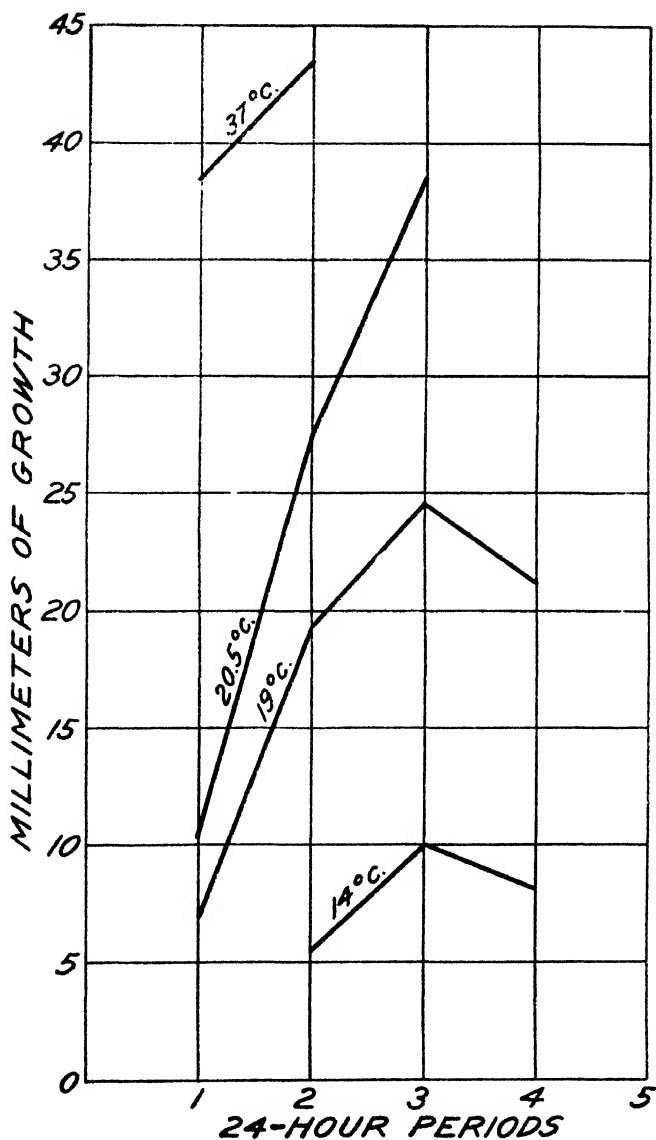


FIG. 18 —Graph showing the increase of growth of *Rhizopus delemar* for consecutive 24-hour periods



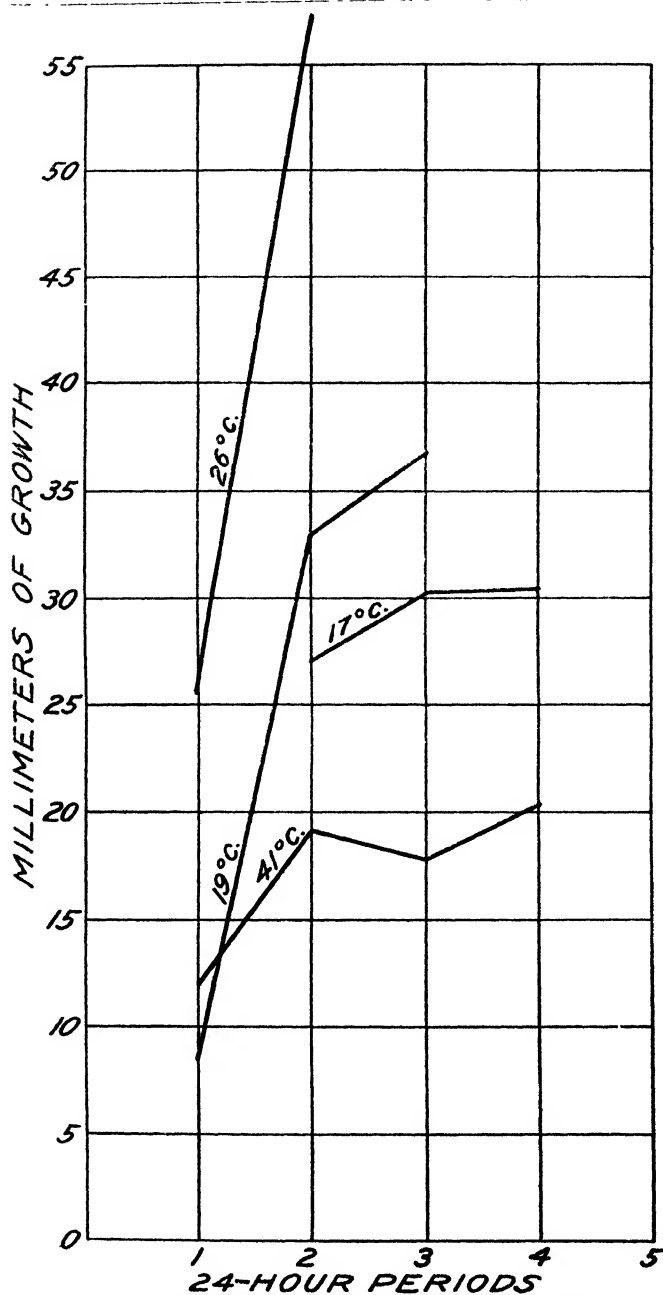


FIG. 19.—Graph showing the increase of growth of *Rhizopus nodosus* for consecutive 24-hour periods.

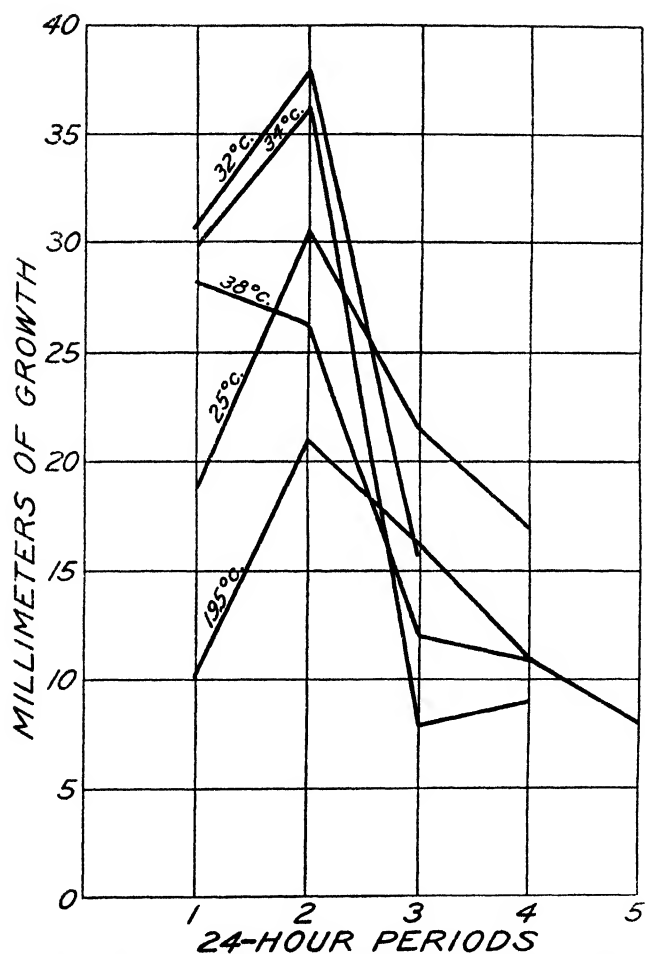


FIG. 20.—Graph showing the increase of growth of *Rhizopus oryzae* for consecutive 24-hour periods.

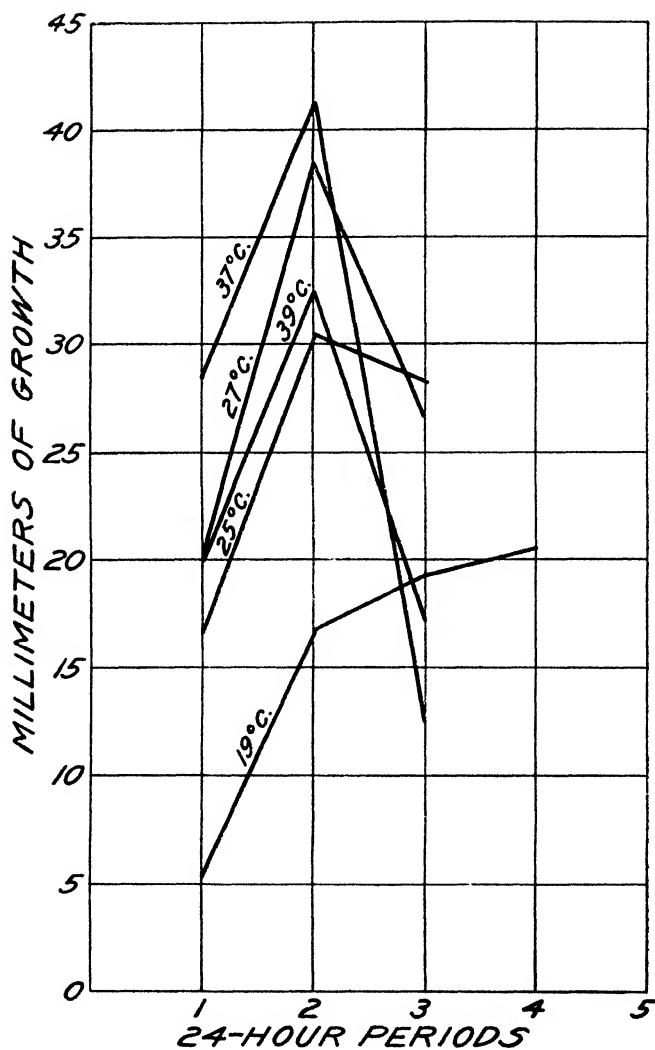


FIG. 21.—Graph showing the increase of growth of *Rhizopus arrhizus* for consecutive 24-hour periods.

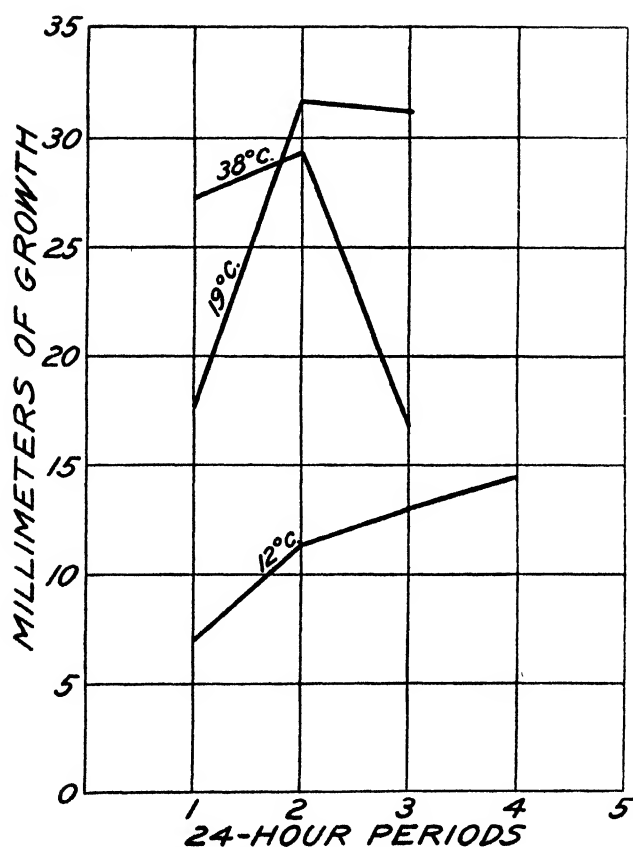


FIG. 22.—Graph showing the increase of growth of *Rhizopus maydis* for consecutive 24-hour periods.

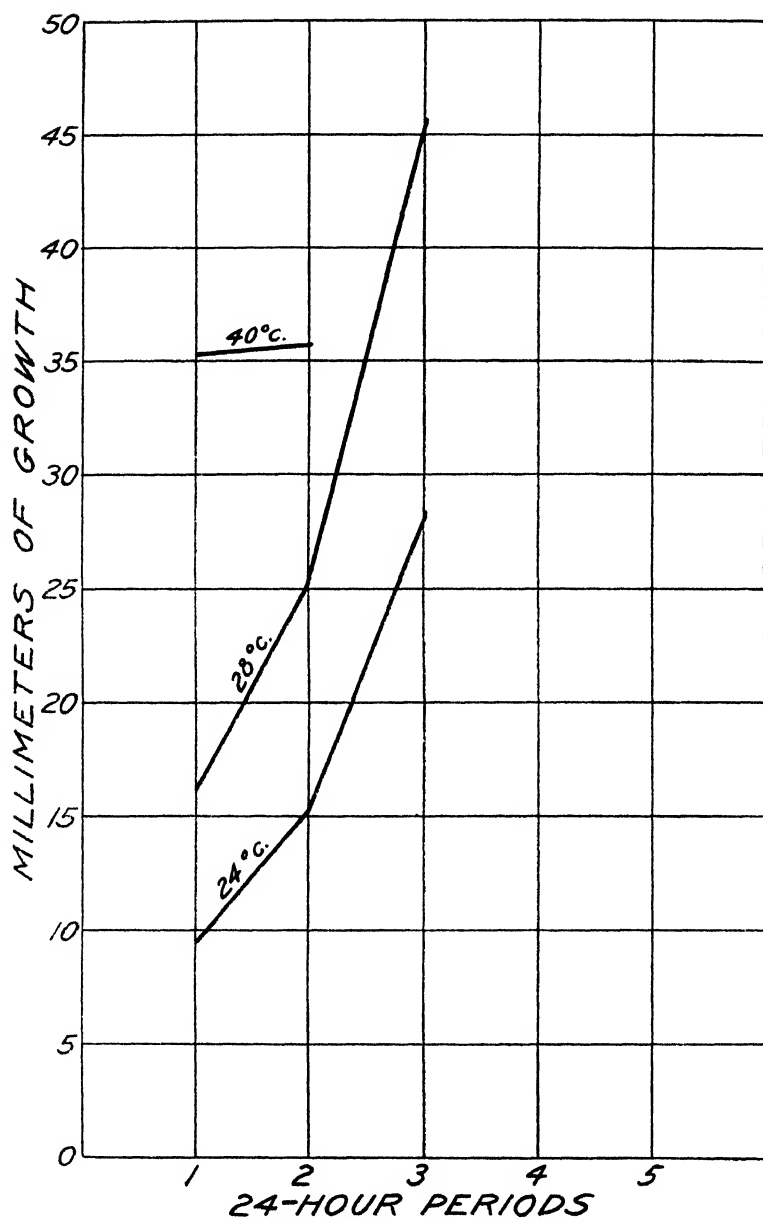


FIG. 23 —Graph showing the increase of growth of *Rhizopus chinensis* for consecutive 24-hour periods.

# INFLUENCE OF TEMPERATURE ON FRUITING.

It has been repeatedly demonstrated by different investigators that the range of temperature at which fungi will sporulate is not coextensive with that at which they will grow. It has been proved that the various species of *Rhizopus* often cause considerable damage to vegetables and fruits. Although under suitable environmental conditions the causal organism can spread from host to host by means of their mycelium, their distribution is accomplished largely by means of the spores. It was therefore thought that information regarding the effect of temperature on sporangia formation, especially at the lower temperatures, might be of considerable practical value. The data accumulated during these studies are given, together with the data on the effect of temperatures on spore germination and growth, in Table I. In all cases those temperatures at which no growth took place and those at which some growth was made are listed in the columns headed "absent" and "present," respectively. The results show that fruiting of these fungi takes place over a considerable range of temperature. The optimum for some of the species is rather sharp and can be easily determined, while in other cases it extends over several degrees. *Chinensis*, *nigricans*, and *artocarpi*, which showed very sharp optimums for spore germination, fruited about equally well over a considerable range of temperatures. On the other hand, *arrhizus*, *reflexus*, and *nodosus* respond readily to temperature, both in regard to germination and fruiting.

Although *artocarpi* belongs to the low-temperature group, when its optimum is considered it stands among the highest-temperature forms with respect to its minimum for fruiting. *Nigricans* and *reflexus*, two low-temperature forms, also have a low minimum for fruiting; whereas *chinensis*, which has the highest optimum and maximum, has about the same minimum as the intermediate forms. The group consisting of *tritici*, *delemar*, *arrhizus*, *oryzae*, and *nodosus* has practically the same minimum, optimum, and maximum temperatures for both growth and fruiting. Hanzawa (9) found that *delemar* and *nodosus* did not fruit at 37° to 42° and 38° C., respectively. The maximum for *arrhizus* and *chinensis* was found by Hanzawa to be 36° and 38° to 42°, respectively. Of the five species just mentioned, *tritici* and *nodosus* alone formed sporangia at 8° to 10°. Hagem (8) gives 38° as the upper limit for *nodosus* and 36° as the maximum for *arrhizus*.

TABLE I.—Minimum, optimum, and maximum temperature in degrees centigrade for spore germination, mycelial growth, and fruiting for 11 species of *Rhizopus*

Species.	Spore germination.				
	Minimum		Optimum	Maximum	
	Absent.	Present.		Present	Absent
<i>artocarp</i>		1 5	26 to 29	33. 5	34 5
<i>nigricans</i> . . .		1. 5	26 to 28	33	34
<i>reflexus</i> . . .		1. 5	30 to 32	36 6	38
<i>microsporus</i>		1 5	26 to 28	33	34
<i>tritici</i> . . .		1. 5	36 to 38	44	45. 5
<i>delemar</i>	7	8. 7	36 to 38	44	45. 5
<i>nodosus</i> . . .		1. 5	36 to 38	44	45. 5
<i>oryzae</i>	7	9	36 to 38	44	45. 5
<i>arrhizus</i>		1 5	36 to 38	43 6	45. 5
<i>maydis</i>					
<i>chinensis</i>	8 5	10	43 to 45	51	52

Species.	Mycelial growth				
	Minimum		Optimum.	Maximum	
	Absent	Present		Present	Absent
<i>artocarp</i>	7	9	26 to 28	32	33
<i>nigricans</i> . . .	1 5	6 5	23 to 26	30 7	31. 5
<i>reflexus</i> . . .		1 5	26 to 28	33	34
<i>microsporus</i>		1 5	25 to 27	30	32
<i>tritici</i> . . .	1 5	6. 5	33 to 35	42	45 5
<i>delemar</i>	7 5	9 8	32 to 34	41	45. 5
<i>nodosus</i>	1 5	6 5	33 to 35	41. 5	45. 5
<i>oryzae</i>	7. 5	11	31 to 34	42	45. 5
<i>arrhizus</i>	1 5	7 4	32 5 to 35. 5	40. 9	44 9
<i>maydis</i>	1. 5	7 4	30. 5 to 32. 5	40	44 5
<i>chinensis</i>	7 5	10 4	37. 5 to 40 5	49	51

Species	Fruiting				
	Minimum.		Optimum	Maximum	
	Absent	Present.		Present	Absent
<i>artocarp</i> . . . . .	12. 5	17	22 to 27	30	32
<i>nigricans</i> . . . . .	7	10	23 to 28	30	32
<i>reflexus</i> . . . . .	7. 6	10. 6	26 to 28	31	32. 5
<i>microsporus</i> . . .	(1)	(1)	(1)	(1)	(1)
<i>tritici</i> . . . . .	12	16. 5	32 to 34	40	45
<i>delemar</i> . . . . .	12	15	32 to 34	38	40
<i>nodosus</i> . . . . .	12. 5	17	32 to 35	37	38. 5
<i>oryzae</i> . . . . .	12	15	32 to 36	37	40
<i>arrhizus</i> . . . . .	11	15	32 to 34	37	40
<i>maydis</i> . . . . .					
<i>chinensis</i>	12	16	35 to 40	45	50

(1) Not tested.

## CERTAIN ENVIRONMENTAL FACTORS INFLUENCING GERMINATION AND GROWTH

## TEMPERATURE AT WHICH THE SPORES ARE PRODUCED

Wiesner (22) found that the temperature at which the mycelium of *Penicillium glaucum* Link developed influenced the time required for the germination of the spores. For example, he showed that spores which matured at 14° C. germinated more quickly at 3° than those which had developed at 3°, while the spores that were produced at 22° germinated more slowly than those produced at 14°. The influence which the growing temperature of *Rhizopus nigricans* has on the germination of its spores has been studied to some extent by the writers. Stock cultures were grown for 7 days on Irish potato agar in Erlenmeyer flasks at 16°, 24°, and 27°. Spore suspensions were prepared in portions of the same solution. The hanging drops were prepared as previously described and incubated at 26°. The spores grown at 16°, 24°, and 27° formed germ tubes as long as the spores in 2 hours, in 2 hours and 5 minutes, and in 2 hours and 25 minutes, respectively. Similar tests were made with *delemar* with the following results. Spores produced at 20.5°, 27.5°, 31.5°, and 36.5° germinated at 36.5° after 3 hours and 45 minutes, 3 hours and 50 minutes, 4 hours and 45 minutes, and 7 hours + (no germination within 7 hours but some later), respectively. Tests with some of the other species showed a similar tendency—that is, spores which were produced at the lower temperatures germinated more quickly than those from cultures grown at higher temperatures. It was thought that possibly the difference in the rate of germination of the spores produced at different temperatures was due to a difference in age. The physiological activities of the organism are admittedly more rapid at the higher temperatures, the spores therefore being produced more quickly and probably aging more rapidly. In order to determine whether the difference in the rate of germination of spores produced at different temperatures might have been due to the age of the spores in the foregoing experiments, another test was made in the following manner. Fifteen 100-cc. Erlenmeyer flasks, each containing 30 cc. of Irish potato agar, were inoculated with a loop of a suspension of *nigricans* spores. Five of these flasks were then held at each of the following temperatures 10°, 20°, and 26°. The time required for the development of the sporangia was noted in each case, and germination tests were made from time to time. The tests were conducted at 25.5°. The spores from cultures which had been fruiting for 3, 5, 8, and 12 days at 20° and 26° germinated in 3¼ hours. One flask held at 26° became contaminated and was discarded. The final test for the spores produced at 20° was made after 20 days, and the time required for germination was the same as when the other tests had been made. The spores produced at 10° when 3, 5, and 15 days old required only 2¾ hours for germination to start. The other two cultures failed to fruit. These experiments show first that spores produced at the lowest temperature (10°) germinated 30 minutes earlier than those formed at 20° and 26°, and second that the age of the spores, at least up to 20 days, did not influence the time necessary for the beginning of germination. It is quite evident from these results that it is important to grow the stock cultures used in comparative experiments at the same temperature.



Experiments were next conducted to determine whether the influence of temperature on the germination of the spores is reflected in the rate of mycelial growth. Irish potato agar contained in 5 Petri dishes was inoculated with a loop of a spore suspension from cultures of *tritici* and *delemar* grown at 20.5°, 27.5°, 31.5°, and 36.5° C. The plates were held at 34° to 35°. At the end of 24 and 48 hours the growth of *tritici* had reached a diameter of 50.6, 50.8, 48, and 45.8 mm. and 84, 92, 82, and 79.5 mm., respectively. The growth of *delemar* measured at the end of 24 and 48 hours 46, 42, 40.5, and 34.6 mm. and 79.5, 81.4, 77, and 78.8 mm., respectively. In general *tritici* showed a slight decline in the rate of growth when the spores were grown at the two highest temperatures. *Delemar* showed a more marked difference at the end of the first 24 hours, there being a gradual decrease from 46 to 34.6 mm.; but this was largely overcome during the next 24 hours, as shown by the fact that there was less than 1 mm. difference between the two extreme temperatures. The temperature at which the spores are produced undoubtedly influences their rate of germination and the early period of the growth of the mycelium developed therefrom. The evidence seems to point to the fact that different species react differently in this respect, some being much more sensitive to small changes in temperature than others.

#### CULTURE MEDIA

Tests were also made to determine to what extent, if any, different media would affect the rate of germination of *nigrkans* spores. The media used were sweet potato decoction, beef bouillon, distilled water, string bean agar, Irish potato agar, and a synthetic agar. The media were placed on cover slips, spores from a single culture were sifted on, and the slips were then inverted over glass rings on slides in the usual manner and incubated at 26° C. The time necessary for germination in each case was as follows. Sweet potato decoction, 2 hours; beef bouillon, 2 hours and 25 minutes; distilled water, 3½ hours; string bean agar, 2 hours and 10 minutes; Irish potato agar, 2 hours and 20 minutes, and synthetic agar, 3 hours. Sweet potato decoction proved the best solution tried, and string bean the best agar. This experiment was duplicated on different days, using spores from a different culture with very similar results. It seems clear that *Rhizopus* spores require something more than water for good germination, since in distilled water a much smaller percentage of the spores germinated, and the germ tubes produced were considerably more slender than those supplied with nutrients. Tap water was found by comparative tests to be a less favorable medium for the germination of these spores than distilled water.

In order to test the effect of the substrate upon the rate of mycelial growth, plates were prepared in the usual manner, using string bean and Irish potato agars. The plates were inoculated with a platinum loop of the same suspension of *nigrkans* spores in sterile distilled water. Five plates of each agar were used at each temperature. Measurements of the diameters of the mycelial disks were made in the usual way, and an average taken of the growth on each medium at the different temperatures. The results are given in Table II.

TABLE II — *Comparative rate of growth of Rhizopus nigricans on string bean and Irish potato agars at different temperatures*

Temperature °C.	24 hours		48 hours.	
	String bean agar	Irish potato agar	String bean agar.	Irish potato agar.
	Mm	Mm	Mm	Mm
33	0	0	0	0
25.7	41.4	23.8	92	46.6
23.3	40.4	22	92	42.2
19.4	22.4	13.8	73.8	39
12.9	7.4	6.2	48	24
11.0	0	0	0	7.8
10.7	0	0	0	0

The data show that for the most part *nigricans* grows nearly twice as fast on string bean agar as on Irish potato agar. The plates of bean agar at 25.7° C. after 48 hours were completely covered, and it was evident that the diameter of the growth would have been somewhat greater had it not come in contact with the edge of the dish. The plates at 23.3° were just covered. The difference in the amount of growth on the two agars at 11.0° was very slight but yet apparent. The cardinal temperatures on the two media appear from the result of these experiments to be about the same. However, to determine this point with certainty, much closer temperatures than those used by the writers would have to be employed.

#### DEXTROSE

While studying the effect of different culture media upon growth it was observed that *nigricans* grew at 30° C. on a synthetic agar but failed to do so on the other media used.

It was thought that perhaps the differences in the acidity of the media might account for this shifting of the maximum temperature. Hence, an experiment was prepared in which Irish potato and beef agars made up to the same H-ion concentration as the synthetic agar, were used, together with synthetic agar as a control. The fungus, however, failed to grow on either of the modified agars at 30° C., but did grow on the synthetic agar. This experiment demonstrated that the H-ion concentration of the media was not the cause of the shifting of the maximum temperature of this fungus.

The synthetic agar differed from the other agars in sugar content as well as acidity, and for this reason tests were made to determine the effect of the addition of different quantities of dextrose to the media. Irish potato and beef agars were prepared to contain roughly 1, 5, 10, 15, and 20 per cent and carrot agar 1, 5, and 10 per cent dextrose. Ten plates were prepared in the usual way with these as well as with unmodified string beans, corn meal, and sweet potato agars. Plates of synthetic, Irish potato, beef, and carrot agars were prepared in the same manner and held as controls. All the plates were held at 30° C. for 48 hours, when the diameter of the growth was measured. This experiment was repeated several times. The average figures for all tests are presented

in Table III. In the cases where no growth is recorded the spores usually had germinated and formed short germ tubes but never formed a spot which spread appreciably beyond the area covered by the drop of spore suspension. Exposure for 48 hours to a temperature at which no growth took place was sufficient to kill the fungus, as was shown by the fact that it failed to make a further growth when held for 24 hours at a temperature suitable for its development.

TABLE III — Comparison of the growth made by *Rhizopus nigricans* at 30° C. on different agars with and without dextrose added

Agar used.	Not modified	With addition of dextrose				
		1 per cent.	5 per cent.	10 per cent.	15 per cent.	20 per cent.
Irish potato ..	No growth	No growth	Mm. 7	Mm. 49	Mm. 59	Mm. 57
Beef ..	do	do	No growth	30	52	54
Carrot. ....	do	do	do	23		
String bean...	do					
Corn meal ..	do					
Sweet potato ..	do					
Synthetic ..	<sup>1</sup> 47					

<sup>1</sup> Contained 20 per cent dextrose

The table shows that the strain of *nigricans* used (4652) did not grow at 30° C. on any of the unmodified agars tried except synthetic agar, which contained 20 per cent dextrose as originally prepared. Upon the addition of 10 per cent or more of dextrose to Irish potato, beef, or carrot agar growth did take place at this temperature. The addition of more than 15 per cent of sugar seemed to have little effect.

After it was determined that the maximum temperature for growth was raised by the addition of dextrose to the medium, experiments were conducted to learn the extent of this change. A loop of the same spore suspension was placed in the center of each of 20 Petri dishes, one-half of which contained unmodified Irish potato agar and the other half some of the same agar to which 20 per cent dextrose was added. The plates were then held at 31° C. Observations after 48 hours showed that the spores had not germinated on the unmodified agar while an average growth about 5 mm. in diameter had been made on the agar with 20 per cent dextrose added. A slight additional growth was made after 48 hours longer on the agar with sugar added. The results of these experiments seem to justify the conclusion that the maximum temperature for the growth of this organism on Irish potato agar is raised from 1° to 1.5° C. by the addition of 20 per cent dextrose to the medium.

That the presence of dextrose in the medium influences the minimum temperature was demonstrated by placing a loop of spore suspension in the center of each of 20 plates of Irish potato agar to one-half of which 20 per cent dextrose was added. The plates were then placed at a temperature of from 0.8° to 2° C. The spores had not germinated at the end of 17 days on the unmodified agar. On the other hand, germ

tubes of from three to five times as long as the diameter of the spores had formed on the agar containing 20 per cent dextrose. The differences on the two agars seem to indicate that the presence of 20 per cent dextrose in Irish potato agar lowers slightly the minimum temperature for germination.

To determine whether the optimum would be changed by the addition of dextrose, plates of each of the agars were prepared and placed at temperatures of 23, 26.5, 27.8, 28.7, and 30° C. Measurements of the diameters of the mycelial growths after two days at 23, 26.5, 27.8, 28.7, and 30° on the unmodified agar were 42, 65.6, 61, 51.8, and 0 mm., and on agar with 20 per cent dextrose added 66.4, 87.8, 92, 92, and 83.2 mm., respectively. These figures show that the optimum for growth on the unmodified agar was 26.5°, while that on the agar containing 20 per cent dextrose probably lay between 27.8 and 28.7°. The measurements of the diameters of the growths at 26.5, 27.8, and 28.7° after 24 hours were 26, 36, and 34 mm., respectively, showing that the optimum was nearer 27.8° than 28.7° but nearer the latter than 26.5°, and was raised 1.5° to 2°.

These experiments show rather conclusively that the presence of dextrose in Irish potato agar shifts the cardinal temperatures of this strain of *Rhizopus nigricans*. Thiele (21) also found that the addition of dextrose to glycerine and formic acid changed the maximum temperatures for the growth of one species of both *Penicillium* and *Aspergillus*. He observed that dextrose added to glycerine and to formic acid caused a shifting of the maximum temperatures for the growth of *Penicillium* of 5° and 4° C., respectively. The temperature maximums for the germination of *Aspergillus* spores and for mycelial growth were raised about 2° and 3°, respectively, by the addition of different amounts of dextrose. On the other hand the maximum for total growth was raised about 2° when growing on glycerine and lowered 3° on the higher concentrations of formic acid. He concluded that the nutritive value of a substance is correlative to a certain extent with the temperature. Similarly, Bruderlein (3) found the growth of *R. maydis* to be retarded on potato, almost arrested on carrot, and prohibited entirely on agar at 42°. Peltier (15) showed that the minimum temperature for the growth of *Pseudomonas citri* Hasse when grown in beef bouillon and on soluble starch agar is slightly higher than on cooked potato cylinders, its maximum being slightly higher in the former than on the two latter media. Likewise, Goss (7) found that *Fusarium trichothecioides* Wollenw. made its best growth on a synthetic solution at 25° and no growth at 5°. On the other hand, the optimum lay between 15° and 20°, and a weighable growth was made at 5° when this fungus grew on Link's potato extract.

Although it has been demonstrated by the writers and others that the cardinal temperatures of some fungi vary with the nature of the substrate, the principles underlying this phenomenon have never been explained. Thiele (21) attributes the shifting of the maximum temperature of the fungi with which he worked to the different nutritive value of the substrate at different temperatures. It may be in the case of the writers' experiments that the dextrose when present in considerable concentration (10 per cent or over) was more available and hence supported growth at a higher temperature than when it was present only in small amounts. It is a well-known fact that increasing the molecular concentration of a solution raises its boiling point and lowers its freezing point. It is not known whether or not the increased concentration of the dextrose in the substrate causes a sufficient increase in the molecular

concentration of the cell sap, thereby protecting the protoplasm of the cell against coagulation by the heat to account for the raising of the temperature maximum  $1^{\circ}$  to  $1.5^{\circ}$  C. The high concentration of sugar in the substrate may also act to lower the water content of the cells of the fungus and thereby increase the concentration of the cell sap which would tend to raise its maximum and lower its minimum. However, the true explanation of this phenomenon must await the accumulation of more experimental data.

#### DISCUSSION

It is apparent from the foregoing curves and tables that the cardinal temperatures for spore germination, growth, and fruiting of the fungi studied vary somewhat. In general the spores will germinate at a temperature too low for mycelial growth. A higher temperature is required for fruiting than for growth. The same thing holds true for the optimum temperatures, although not to the same extent. The optimum for germination is always higher than for growth and fruiting, while in most cases the optimum for fruiting is about the same as that for growth. The slight differences shown in Table I may be due to the fact that these figures give the optimum temperatures tried rather than the true optimum. The latter probably does not differ from the former more than a plus or minus  $1$  or  $2^{\circ}$  C., since the temperature of the incubators usually differed only from  $2$  to  $5^{\circ}$ , the maximum differences being between the extreme temperatures. The optimum temperature for fruiting is often not so well defined as that for growth, and the latter less so than for spore germination.

In each case there is a gradual gradation in the maximum from that at which the spores will germinate to that at which fruiting will take place, the maximum for growth being about midway between that for germination and that for fruiting. This fact may be explained in part at least in two ways; first, the spores germinate quickly, probably before the heat acts injuriously; and second, the spores are probably less sensitive to heat than are the germ tubes. Spores which before germination are uninjured by the heat may be readily killed after they germinate. At a slightly lower temperature growth becomes evident but ceases after a time, and the mycelium is killed before sporangia are formed.

The time factor is of greater importance at the minimum than at any other temperature. At low temperatures, germination is greatly retarded, the growth being often so sparse that no measurable felt is produced within the time limit of these experiments.

The genus *Rhizopus* includes some of the best known, most widely distributed and most destructive of the fungi. *Nigricans* is perhaps the most destructive, since it attacks a wide range of hosts under widely different conditions, being especially destructive to tomatoes, strawberries, and sweet potatoes. However, this species is somewhat limited in its field of destruction by temperature. For example, in these experiments its spores were killed by a comparatively short exposure at a temperature of  $34^{\circ}$  to  $35^{\circ}$  C., and it made a very slow growth at  $6.5^{\circ}$  ( $35$  mm. in 30 days) and no appreciable growth at  $1.5^{\circ}$  after 30 days. Although some variation in its response to temperature on different substrates may be expected, there is little doubt that decay of fruits and vegetables by this organism can be prevented or retarded by proper cold storage. A temperature as high as  $7^{\circ}$  or thereabouts will prevent fruiting for several days and hence retard the spread of this fungus. It

must be kept in mind, however, that other species may be destructive at temperatures which are not favorable for the development of *nigricans*, although at the present time little is known regarding the distribution and destructiveness of the other species under natural conditions in the United States

# SUMMARY

(1) The effect of temperatures on the spore germination, mycelial growth, and fruiting of 11 species of *Rhizopus* has been studied, and the results are presented in this paper.

(2) These 11 species fall into three groups as regards their response to temperature. *Chinensis* has a temperature maximum and optimum several degrees higher than any of the other species and hence can be easily separated from them; *nigricans*, *microsporus*, *reflexus*, and *artocarp*i make up a group having a low optimum and maximum; while the 6 remaining species, namely, *tritici*, *nodosus*, *delemar*, *oryzae*, *arrhizus*, and *maydis* constitute an intermediate group.

(3) In general the spores will germinate at a temperature above the maximum for continued growth.

(4) The optimum for germination for all the species is higher than for growth and fruiting, while in most cases the optimum for fruiting is about the same as for growth.

(5) The temperature at which the spores are produced influences to some extent their rate of germination and the early period of the growth of the mycelium developed therefrom. Spores of *nigricans* produced at 10° C. germinated in 30 minutes less time than those formed at 20° and 26°. Spores of this species from different cultures grown under like conditions germinated equally well, regardless of age, up to 20 days.

(6) Spores of *nigricans* germinate in a considerably shorter time in a nutrient solution than in water. Sweet potato decoction proved to be the best liquid and string bean agar the best solid medium tried. In the comparative tests made this fungus grew nearly twice as fast on string bean agar as on Irish potato agar.

(7) The presence of 20 per cent dextrose in Irish potato agar changed the cardinal temperatures of the strain of *nigricans* studied from 1 to 2° C.

(8) *Nigricans*, which seems to be the most destructive member of this genus, under natural conditions is somewhat limited in its field of destruction by temperature. The spores were invariably killed at 35° C., and growth was very sparse and slow at 6.5°. At 1.5° no appreciable growth was made on Irish potato agar in 30 days.

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concentration of 125 parts per million is just about the concentration producing the best growth under these conditions, as shown by other work; and as will be seen later, there was very little likelihood of the plant absorbing very much more plant food than was actually needed for its development.

Beginning with the fifth day after the seeds were placed in the solution, on every third day enough plants for an analysis were drawn from the pans, care being taken to draw some plants from each pan to equalize variations in the cultures as much as possible. When one of the culture pans fell below the normal it was discarded. In this way a representative set of plants could be withdrawn each time. The analyses of the plants, together with that of the original seeds, are shown in Table I, the results being expressed on the basis of 100 plants.

TABLE I.—Analyses of 100 wheat seedlings, grown in nutrient solutions, at different stages of growth

No.	Stage of growth.	Dry weight	N	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>
		Gm.	Gm	Gm.	Gm
1	Original seeds.....	2.14	0.0490	0.0295	0.0210
2	5 days old.....	1.89	.0565	.0272	.0184
3	8 days old.....	2.05	.0742	.0489	.0250
4	11 days old.....	1.82	.0812	.0869	.0308
5	14 days old.....	1.95	.0924	.1203	.0336
6	17 days old.....	2.30	.1160	.1311	.0570
7	20 days old.....	3.04	.1410	.1466	.0740
8	23 days old.....	3.50	.1946	.1610	.1020
9	26 days old.....	4.64	.2110	.2018	.1220

It will be noticed that there is a falling off in dry weight, in potash, and in phosphoric acid, but not in nitrogen, up to the fifth day. The loss in weight is, of course, due largely to the decomposition of starch and sugar of the seeds and to the evolution of carbon dioxide. This loss often amounts to as much as 40 per cent of the dry weight before enough plant food and carbon dioxide are absorbed to balance the loss. Many experimenters with wheat seedlings, when using dry weight as a criterion, fail to realize the fact that in the first stage of growth they are dealing with a diminishing quantity. The loss of potash and phosphoric acid always takes place at the beginning of germination, due to the exudation of these plant foods from the seed. It might be added that these very salts that are exuded from the seeds are absorbed by the seedling in a few days or as soon as the radicle has become sufficiently developed. There is usually little exudation of nitrogen for the reason that the nitrogen of the seed exists in organic combinations, protein, and is not readily dissolved out by water.

These results when plotted are represented by figure 1.

By cutting the curves through any particular date it seems possible to determine the relative demand of the plant at that age for the three important plant foods. It is also evident that the relative demand for food changes very rapidly as the plant develops. Beginning about the fifth day, when the plants begin to feed, the curve for potash rises very rapidly. The little seedling awakes to life with a ravenous appetite for potash, out of proportion to other plant foods. When the seeds are moistened and warmed preparatory for germination, the potash which

is stored up in the seed, and which is fairly evenly distributed through it, begins to move very rapidly toward the end containing the germ or embryo. In the germination, when the embryo has broken through the bran coating and is just large enough to get hold of with the thumb and finger nails, it contains about 50 per cent of the total potash of the seed. The seed does not contain enough potash to satisfy the demand of the little seedling, so it begins early to feed heavily on the nutrient of the solution. Probably, in the natural course of life, the very first food absorbed by the little seedling is potassium. The absorption of nitrogen is steady and comparatively uniform, so after 18 or 20 days the curves for potash and nitrogen cross each other. The absorption of phosphoric acid is regular also.

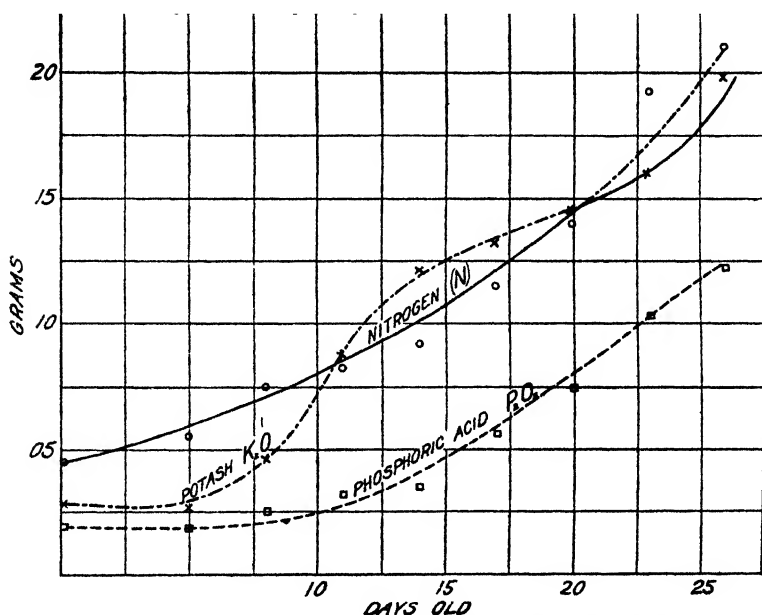


FIG. 1.—Graph illustrating the absorption of plant foods from nutrient solutions by wheat seedlings at different stages of growth.

Without submitting all the data, the curves for a duplicate determination, made about one month later, are given in order to bring out the crossing of the curves of nitrogen and potash (fig. 2).

The data presented give an idea of the nutrition of a plant when feeding full time and under favorable conditions. But a plant in nature does not necessarily have ideal conditions in which to grow. A low moisture content of the soil may temporarily put a stop to nutrition or the fluctuation of plant food, particularly nitrates, in the soil solution may also slow down, or even stop, absorption. It is, therefore, important to know what percentage of the time is actually necessary for absorption and whether a plant can absorb enough plant food in one period of time to last it over another.



## DEMAND OF WHEAT PLANTS GROWN IN NUTRIENT SOLUTIONS FOR FRACTIONAL PARTS OF A DAY

Seedlings were placed in culture pans containing a full nutrient solution of a concentration of 125 parts per million each of nitrogen, potash, and phosphoric acid, as before described, and allowed to feed for fractional parts of the day. At the end of the period allotted to each lot to remain in the nutrient solution, the disks with the seedlings were taken up and washed off and placed in distilled water for the remainder of the day. This process was repeated daily for 10 days, and the plants then analyzed for nitrogen, potash, and phosphoric acid. In Table II is

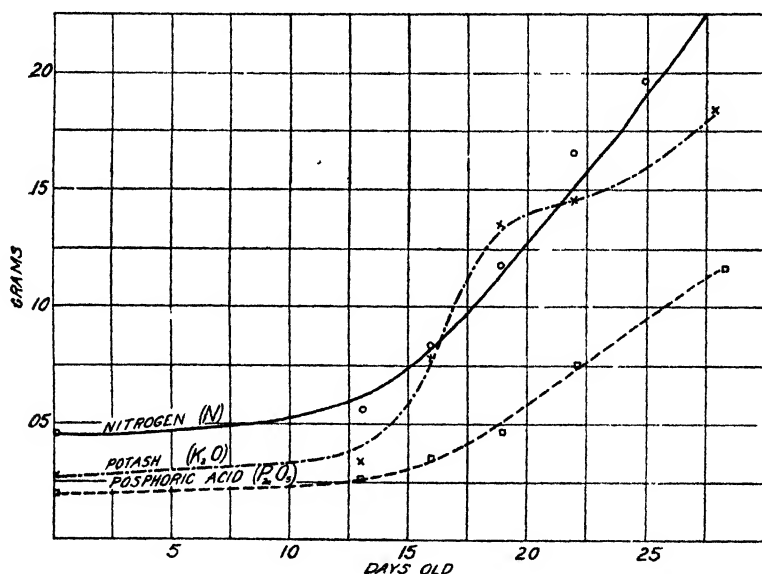


FIG. 1.—Graph illustrating the absorption of plant foods from nutrient solutions by wheat seedlings at different stages of growth.

shown the number of hours each day the plant remained in the nutrient solution and the quantities of plant food absorbed.

TABLE II.—Analyses of 100 wheat seedlings feeding fractional parts of the day for 10 days

No	Hours per day in nutrient solution.	Dry weight	N	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>
		Gm.	Gm.	Gm.	Gm.
1	Control o. . . . .	5.32	0.0770	0.0318	0.0420
2	1. . . . .	5.15	.0882	.0838	.0560
3	2. . . . .	5.60	.1022	.1047	.0750
4	4. . . . .	5.20	.1120	.1366	.0840
5	6. . . . .	5.80	.1540	.1862	.0960
6	8. . . . .	5.30	.1680	.1940	.1040
7	12. . . . .	5.73	.2072	.1858	.1060
8	24. . . . .	6.28	.2352	.2475	.1190

It will be seen by referring to Table II and figure 3 that the absorption of plant food for 1 hour per day, and other fractional parts, is out of proportion to what might be expected if time alone governed absorption. For example, subtracting the amount of potash in the original seed, or in the control, from the analysis of the plants grown 1 hour a day in the nutrient solution, we get 0.0520 gm. of potash actually absorbed by the plants during that time. In the same way by subtracting the control from those grown 24 hours a day in the nutrient solution,

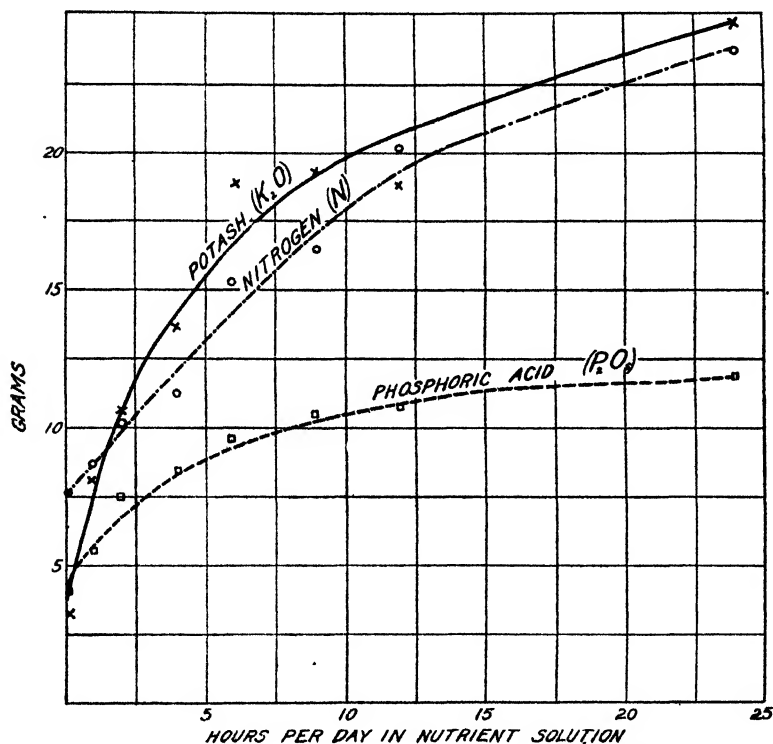


FIG. 3.—Graph illustrating the absorption of plant foods by wheat seedlings when grown for fractional parts of the day for 10 days in nutrient solutions

we get only 0.2157 gm. of potash actually absorbed in 24 hours. If the rate of absorption of the plants kept in the nutrient solution 1 hour a day had been maintained in the plants kept in 24 hours a day, we would have had an absorption of 1.248 gm. instead of 0.2157 gm. In other words, we get over five times as much potash absorbed in the 1-hour periods as might be expected if time alone governed absorption. This phenomenon is true with other plant foods; the curves rise very abruptly from the start and flatten out as the time increases. The demand or desire for food seems to accumulate until the food becomes available; then an abnormal rate of absorption takes place. The figures, when plotted, are represented in figure 3.

The abrupt rise of the potash curve is noticeable, showing the demand for potash in the seedling was out of proportion to that for other plant foods. The cumulative demand, brought out in this experiment, seems to be true, not only for fractional parts of the day but for much longer periods.

#### DEMAND OF WHEAT PLANTS AFTER BEING HELD IN DISTILLED WATER FROM 2 TO 17 DAYS

Seven culture pans of soft wheat were germinated and grown in distilled water for 4 days. One pan was then transferred to a nutrient solution of 125 parts per million each, nitrogen (N), potash ( $K_2O$ ), and phosphoric acid ( $P_2O_5$ ), while all the others were kept in distilled water. At the end of 3 more days a second pan was placed in the nutrient solution, and this process continued every 3 days until six of the lots had been taken from distilled water and placed in nutrient solution. They were then allowed to grow for 2 more days, when they were taken down and analyzed. Thus the first pan had been feeding from a good nutrient solution for 17 days, while the last pan had been feeding only 2 days. The last pan having been grown in distilled water for 15 days had no increase in growth over the control when placed in the nutrient solution. The results of the analyses are shown in Table III.

TABLE III.—Analyses of 100 wheat seedlings grown two or more days in nutrient solutions

No.	Days in nutrient solutions.	Dry weight.	N	$K_2O$	$P_2O_5$
		Gm	Gm	Gm	Gm
1	Control 0	6. 28	0. 0840	0. 0613	0. 0400
2	2	6. 60	. 1410	. 1154	. 0587
3	5	6. 52	. 1904	. 1685	. 0750
4	8	7. 12	. 2268	. 2090	. 0813
5	11	6. 80	. 2674	. 2350	. 0973
6	14	6. 80	. 2604	. 2361	. 0908
7	17	7. 00	. 2912	. 2580	. 1440

It will be seen by referring to Table III that with each plant food element the absorption for the 2-day period was out of proportion to that of the 17-day period. By subtracting the quantity of potash in the control, for example, from that of the plants grown for 2 days in the nutrient solution, we get 0.0541 gm. of potash actually absorbed in 2 days. In like manner, by subtracting the control from the 17-day plants, we get only 0.1967 gm. potash actually absorbed in 17 days, when we should get 0.459 gm. of potash if time alone governed absorption. These results plot very well, showing that the demand is fairly regular and cumulative. This experiment was repeated, changing the plants in 2-day periods, with similar results.

If Tables IV and V, showing the rates of absorption of the different plant foods, are studied, other interesting relations are brought out. From Table IV it appears that the absorption of potash for the short period is much more pronounced than the absorption of other plant foods. On the other hand, when seedlings are left in the nutrient solution for a number of days (Table V), the absorption of potash and nitrogen shows a remarkable agreement in the rate. This is brought out in figures 4 and 5.

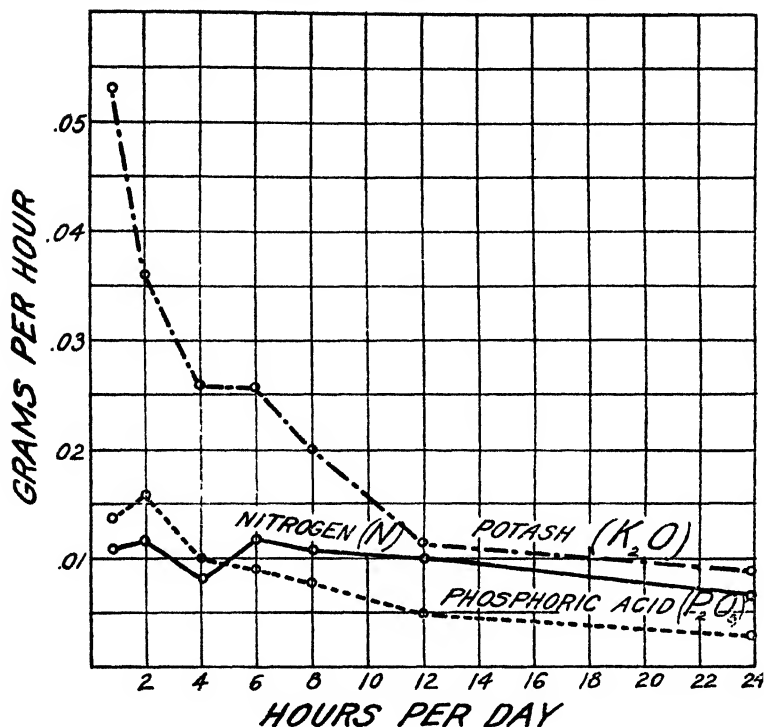


FIG. 4.—Graph illustrating the rate per hour of the absorption of plant foods by wheat seedlings when grown for fractional parts of the day in nutrient solutions

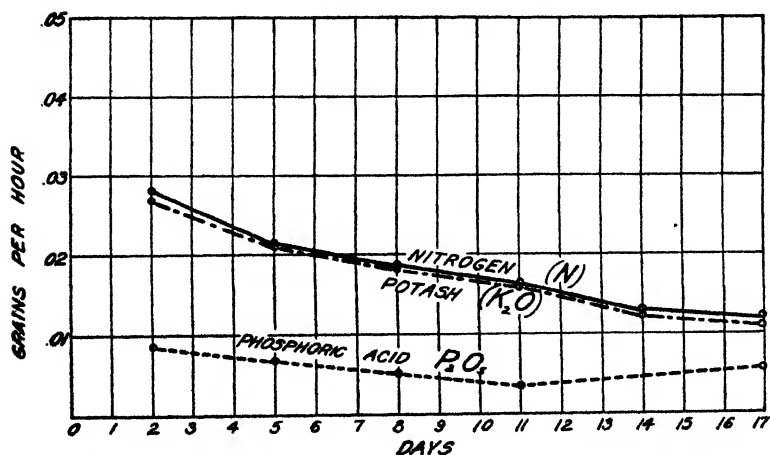


FIG. 5.—Graph illustrating the rate per day of the absorption of plant foods by wheat seedlings when grown for two or more days in nutrient solutions.

TABLE IV.—Rate of absorption of plant foods per hour when wheat seedlings were feeding fractional parts of day

[From Table II]

Hours per day.	Rate of absorption per hour.		
	N	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>
	Gm.	Gm.	Gm.
1..	0.00113	0.00520	0.00140
2	.00126	.00364	.00165
4	.00087	.00262	.00105
6	.00128	.00257	.00090
8	.00114	.00203	.00078
12	.00108	.00128	.00053
24	.00066	.00090	.00032

TABLE V.—Rate of absorption of plant food per day when wheat seedlings were feeding for long periods

[From Table III]

Days in solution.	Rate of absorption per day		
	N	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>
	Gm.	Gm.	Gm.
2....	0.0285	0.02705	0.00935
5...	.0213	.02144	.007
8....	.01785	.01846	.00516
11....	.01667	.01579	.00521
14....	.0126	.01240	.00363
17....	.01210	.01157	.00312

## DEMAND OF WHEAT PLANTS GROWN FOR AN INITIAL PERIOD IN A FULL NUTRIENT SOLUTION AND SUBSEQUENTLY STARVED

The second of the two experiments described above was made in an effort to measure the demands for plant food that might be developed in plants that had been grown in distilled water and had never had any food supply except that contained in the seed. It was thought probable that other results might be obtained if the plants were first fed heavily, then allowed to fast, and the demand brought about by this fast measured in a second feeding period. A separate set was started, and when the plumules had reached a length of about 1 cm. all the culture pans were placed in a full nutrient solution of a concentration of 100 parts per million each nitrogen (N), potash (K<sub>2</sub>O), and phosphoric acid (P<sub>2</sub>O<sub>5</sub>) and allowed to remain in this solution with frequent changes of solution for seven days. All were then placed in distilled water and allowed to stand seven days or more, when No. 4 was again placed in the nutrient solution. After one more day No. 3 was placed in the nutrient solution, and after another day No. 2 was put in the nutrient solution. After one more day all four sets were taken down for analysis. In this way, the plants were all given plenty of food for the first period, then allowed to fast and to develop an appetite during the second period, which was measured in the third period. This is shown in Table VI.

TABLE VI.—Analyses of 100 wheat plants given an abundance of plant food in the first period, placed in distilled water for the second period, and again placed in nutrient solution for two or more days

No.	Days in nutrient solution in third period.	Dry weight	N	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>
		Gm.	Gm	Gm	Gm
1	Control o . . . . .	3. 92	0. 1596	0. 1500	0. 0960
2	1 . . . . .	5. 88	. 2172	. 1998	. 1320
3	2 . . . . .	5. 68	. 2320	. 2506	. 1400
4	3 . . . . .	5. 90	. 2605	. 2670	. 1380

By subtracting the nitrogen in the control, 0.1596 gm., from the nitrogen in the 1-day plant (No. 2), 0.2172 gm., we get 0.0576 gm. nitrogen absorbed in one day. By subtracting the control from the 3-day plants, we get 0.1009 gm. instead of three times 0.0576 gm., or 0.1728 gm., which we might expect if time alone governed absorption. In the K<sub>2</sub>O column one determination, the absorption in 2 days, seems to be a little out of line and high. From these experiments one would judge that a demand for any of the plant foods can be developed in the plant, that this demand is cumulative, and that it is possible to measure this demand by analytical means.

#### DEMAND OF WHEAT PLANTS GROWN IN NUTRIENT SOLUTION FULL TIME AND ALTERNATE DAYS

As nitrates, and possibly other plant foods, are likely to vary in the soil solution from day to day, it was thought of interest to measure the rate of absorption when nutrients were given at varying intervals—that is, to measure the rate of absorption when cultures were placed in nutrient solution one day and in distilled water the next, and in nutrient solution on the third day and so on, feasting for one day and fasting the next. These seedlings were grown both with an abundance of plant food (100 parts per million each N, K<sub>2</sub>O and P<sub>2</sub>O<sub>5</sub>) and also with a limited amount (10 parts per million). Analyses of the 12-day-old plants are given in Table VII.

TABLE VII.—Analyses of 100 wheat plants grown continuously in nutrient solutions for 12 days, compared with similar series placed in distilled water every other day

No.	Treatment	Dry weight	N	K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>
1	Distilled water control . . . . .	4. 44	0. 0924	0. 0597	0. 0720
2	Nutrient solution 100 p. p. m. full time	4. 68	. 1988	. 2561	. 1080
3	Nutrient solution 100 p. p. m. one day, distilled water one day.	5. 36	. 2100	. 2856	. 1280
4	Nutrient solution 10 p. p. m. full time..	5. 76	. 1932	. 2273	. 1200
5	Nutrient solution 10 p. p. m. one day, distilled water one day.	5. 92	. 1736	. 1940	. 1180

This was repeated several times with similar results. The total quantity of plant food absorbed, when plenty of plant food was present, was greater in the plants that had grown in the nutrient solution only half time than in the plants that had grown all the time in the same nutrient. When a limited quantity of plant food was present, the quantity

absorbed was reduced by removing the plants from the nutrient solution for half the time. But when calculated upon the rate of absorption per day, both the plants in the strong solution and those in the weak had a higher rate when grown only half time in the nutrient solution.

It is somewhat surprising to note that not only the rate of absorption but the general appearance of the plants—color, size, root development, and vigor of growth—is usually better when the plants are grown in a good nutrient solution for one period and in distilled water the next. This is not always the case but seems to be characteristic of plants that are grown during warm weather, when growth and absorption are rapid, and is not so likely to be true of plants grown in cold weather, when growth and absorption are relatively slow. Better looking plants are often obtained when they are kept in the nutrient solution at night and in distilled water during the day.

The experiments here described seem to demonstrate that a demand for plant food may exist within the plant, that this demand may be modified in different ways, and that the demand may be determined by analytical methods. The author has demonstrated the fact that it is possible to go out in the field, to take up a plant and put it in a full nutrient solution, and to determine what plant food it is hungry for by the way it feeds upon the nutrient solution.

#### TRANSFER OF PLANT FOODS WITHIN THE PLANT

The plant seems to feed upon the ions and not upon the molecules, and no plant seems to require a base and an acid in exactly the proper proportion to form a salt. It has been shown by Breazeale and Briggs<sup>2</sup> that a plant is even particular as to the kind of ion that it absorbs. Plants that had a high demand for potassium when placed in a solution of orthoclase were unable to feed upon the dissolved potassium. The solution was dilute, it is true, but not so dilute as to prevent the absorption of potassium. It is probable that the potassium existed in the solution as a double ion in combination with aluminum. The plant did not need and could not utilize the aluminum and therefore would not take up either the potassium or the aluminum.

That the transpiration rate has little to do with absorption can easily be shown by placing a bell jar over a pan of cultures, cutting down the transpiration, and measuring the rate of absorption in comparison with controls. Plants will feed just as rapidly when transpiration has thus been reduced to a minimum as they will when transpiring a maximum amount of water.

One can scarcely conceive of a plant feeding upon ions or exercising selective absorption in such a decided way if the transpiration stream or the osmotic concentration, or any other phenomenon except the specific demand of the plant, dominates the process of absorption.

In the same way, practical field results indicate that all plants do not possess the same ability to feed when placed in competition with each other. We find that if an oat and a mustard plant be grown in the same pot with a very limited supply of nitrogen, the mustard probably will get nearly all of that plant food and the oat very little. Plants vary widely in their ability to cope with each other when placed in keen competition.

<sup>2</sup> BREAZEALE, J. F., and BRIGGS, LYMAN J. CONCENTRATIONS OF POTASSIUM IN ORTHOCLASE SOLUTIONS OF A MEASURE OF ITS AVAILABILITY TO WHEAT SEEDLINGS. *In* Jour. Agr. Research, v. 20, p. 615-621. 1921.

An experiment with corn and kafir seedlings seems to throw some light upon this subject. Six treatments in duplicate were run, 12 pans in all, with corn and kafir seedlings growing upon the same disks and in the same nutrient solutions. The disks were about 12 inches in diameter and were like those used in the wheat culture work except that the perforations were larger. The corn seedlings were planted upon one half of the disk while the kafir grew upon the other. There were about 50 corn to 300 kafir plants. These seedlings were placed in the following solutions:

- No. 1. Control, distilled water.
- No. 2. 2 parts per million each N, K, and P.
- No. 3. 5 parts per million each N, K, and P.
- No. 4. 25 parts per million each N, K, and P.
- No. 5. 50 parts per million each N, K, and P.
- No. 6. 100 parts per million each N, K, and P.

They were allowed to grow for 17 days. In each pan, 2,500 cc. of solution were used and the experiments were run in duplicate. For convenience the average of the duplicates is given in Table VIII.

TABLE VIII.—*Nitrogen absorbed by corn and kafir seedlings competing in nutrient solutions of various strengths*

No	Strength of solution	Total N added	Nitrogen absorbed	
			By corn	By kafir
		Gm	Gm	Gm
1	Water	0	0	0
2	2 p. p. m.	0.0450	0.0466	<sup>1</sup> 0.0073
3	5 p. p. m.	.1125	.1094	<sup>1</sup> .0091
4	25 p. p. m.	.5625	.2475	<sup>2</sup> .0180
5	50 p. p. m.	1.1250	.3344	<sup>2</sup> .0504
6	100 p. p. m.	2.2500	.3821	( <sup>3</sup> )

<sup>1</sup> Loss

<sup>2</sup> Gain

<sup>3</sup> Poor plants

From this one experiment the indications are that when corn and kafir are placed in keen competition as in solutions containing 2 and 5 parts per million the corn may get all the nitrogen and the kafir little or none. An actual loss of nitrogen is noticed in the kafir in the two lowest concentrations, which might be explained by the exudation of this element into the solution or by the probable error of the experiment. The solutions were well mixed and not stirred while the plants drew out the nitrogen. In places the kafir roots were 6 inches away from the corn and in contact with the nitrates in the solution but, the kafir being a sluggish feeder while the corn was a vigorous feeder, the nitrates seem to have gone to the corn and not to the kafir. The absorption of the small quantity of nitrates was so rapid that it does not seem reasonable to assume that diffusion carried it all to the corn side of the pan. The difference is so marked that one must admit that in this case the kafir had no chance in competition with corn and that the nitrogen must have moved as far as 6 inches in a very short time. If, in a soil at optimum moisture content, the plant is dependent upon the soil grains that touch its roots, this competition is rather difficult to conceive of. Practically speaking, the absorbing surface of the roots of different plants would hardly touch the same soil grains often enough to be of serious consideration.



## MOVEMENT OF PLANT FOODS IN THE SOIL

Ions are mobile and bear plus or minus charges of electricity. It has long been the opinion of the writer that the demand for food originates in the tissues of the plant and is carried to the absorbing surface of the root by means of an unsaturated carbon compound bearing a plus or minus charge. In the case of potassium, for example, the plant protoplasm in a leaf cell may remove an atom of K from a colloidal compound and use it in building up a permanent compound, which is to be one of the final constituents of its tissues. The removal of the atom K, bearing a plus charge, from its colloidal compound leaves that compound or molecule out of equilibrium and with a minus charge. This charge is transmitted, by replacement and not by bodily movement, down through the cells to the root tips and there appears as a minus charge. If potassium chlorid appears in the nutrient solution ionized as a plus K and a minus Cl, the plus ion will be attracted to the negative charge, and a chemical combination will take place, with the formation of a molecule in equilibrium, with respect to plus and minus electricity. The potassium could, in this way, be transported from the absorbing surface of the root to the extreme tips of the plant without a bodily movement through the sap. In the case of nitrates the opposite conditions might prevail. A demand for NO<sub>3</sub> might originate in the tissues and be carried to the root as an unsaturated compound bearing a plus charge. This would be neutralized, for example, by the NO<sub>3</sub> ion of NaNO<sub>3</sub>.

It is possible that the absorbing surface of the root or its walls are actually impermeable to the salts needed in nutrition, and it is possible to assume that food material may be transported from the roots to the rest of the plant without materially affecting the osmotic pressure of the sap. If the food materials are flowing freely in the sap and were it necessary for the plant to remove these materials in the localities where they were needed, it seems probable that there might be times when a high demand and a low supply, or the reverse, might cause considerable fluctuation in the concentration of the sap. As the writer understands it, the sap of plants of like varieties grown in the same localities is fairly constant. The tendency of the plant seems to be to keep its solutions in equilibrium, and the writer can conceive of a plant acting much like the battery of an automobile—the needle of the indicator may register a charge at one instant and a discharge at the next. The plant probably vibrates around the equilibrium point as closely as possible, taking up a plus charge at one time and a minus charge at another. The ordinary plant uses more of the mineral bases than it does of the mineral acids, and the writer is fairly well convinced that in order to maintain equilibrium the plant can absorb otherwise useless acids or bases, use them as ions when necessary, and eliminate them in various ways. With certain plants, if the system is basic, they seem to absorb CO<sub>2</sub> as an acid radical for the purpose of maintaining equilibrium; and in case the system becomes too acid they seem to possess the power of exuding the carbonates as CO<sub>2</sub>. The absorption of calcium by certain plants and its elimination as an oxalate might be explained in this way. The absorption of silica in large quantities may probably be traced to the presence of an excess of basic radicals in those plants. The plants that have this characteristic have usually had waterlogged marsh lands for their habitat, and in the ages of their adaptation have had a large quantity of soluble silica and a small quantity of carbon dioxide at their

disposal and as a matter of necessity have adapted themselves to the former.

The plant seems to feed upon ions, and these ions are certainly mobile. If an ionic movement takes place between the root tips and the rest of the plant, it seems equally reasonable to assume the possibility that a similar movement might exist outside the plant and that the plant might draw its food from relatively long distances.

The potassium concentration of young plants, sometimes having small root systems, often runs very high. The soil solution is relatively low in this element, and water movement and diffusion are negligible factors. The absorbing surface of the root is small, and, assuming that the root is obliged to come in contact with a soil grain before it can draw upon the potassium, it is rather difficult to account for the high potassium content of the plant. In the same way the writer has analyzed many samples of Australian saltbush that ran 8 per cent or more of sodium chlorid upon the basis of their dry weight. These plants had grown in a soil that was very low in sodium chlorid and in a semi-arid climate where the soil moisture was very low for the greater part of the year. It seems hardly possible for a plant with growing habits like that of the saltbush to be able to absorb such quantities of salt as it does, if obliged to feed in the manner usually attributed to plants. It also seems hardly possible to conceive of this plant being forced to absorb sodium chlorid against its will, so to speak, because the salt is carried into the system by the transpiration stream or other agencies. A noticeable feature of this sodium-chlorid absorption is that the sodium (Na) is absorbed in much larger quantities than are necessary for combination with chlorin (Cl) in the formation of sodium chlorid (NaCl). The excess sodium exists in the plant in organic combinations and is broken down into carbonates upon ashing. Evidently there exists in the Australian saltbush a demand for sodium and chlorin, and the demand for sodium is greater than the demand for chlorin. This absorption of the sodium ions in excess of the chlorin, from a soil solution where the source of supply of these ions is sodium chlorid, which is in equilibrium, seems to eliminate the idea of "forceful feeding," as applied to this plant. The high salt content of the plant, with such a low transpiration, would also indicate a wider field of absorption than is usually attributed to it.

If a soil solution is in equilibrium and the salts are ionized and the ions are mobile, if an atom of the K, for example, bearing a plus charge is removed from solution by the root, the writer sees no reason why the position of this ion can not be filled by replacement and the charge carried along through the soil, as it is in a battery, to where the source of supply of potassium exists. If this be true, the plant will not be dependent upon the soil grains that touch the root tips, but it may actually feed at a distance, the distance probably following some well-known physical law. In practical agriculture we, involuntarily, think of the plant as having all the moist soil surface at its disposal. We also think that the water movement in the soil is negligible as far as nutrition is concerned, that the plant has to grow for its water, and that diffusion is also a negligible quantity. With plants that have a limited root system and with the absorbing zone of the root but a small part of the root itself, if we do not attribute to the plant the ability to feed at a distance, we will have to admit that only a small part of the soil is at the disposal of the plant.

## CONCLUSIONS

(1) A demand for plant food may be developed in the tissues of plants, and this demand may be measured by analytical methods.

(2) The demand of the plant seems to be for particular foods, and the effect of an application of plant food as a fertilizer seems to be largely a direct action upon the plant itself and not an indirect action upon some constituent of the soil.

(3) Plants probably feed upon ions, and these ions probably penetrate the root membrane and move through the colloids to the tissues as an electrical charge; therefore the feeding of plants may be looked upon as an electrical phenomenon.

(4) Ions are mobile and may move through the soil solution freely as such; and, this being the case, the plant may not be dependent upon the soil grains that touch its roots for nutrient material but may feed at a distance from the source of supply.

# INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS<sup>1</sup>

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## INTRODUCTION

The pioneer investigations concerning the influence of soil temperature upon cabbage yellows caused by *Fusarium conglutinans* Wollenw. were conducted in Wisconsin by Gilman (8).<sup>3</sup> His experiments were conducted in greenhouse rooms with rather wide fluctuations of air temperature, where the soil temperature could not be held constant for any great length of time. Consequently, Gilman did not determine the complete range of soil temperature for the occurrence of the disease, nor did he inquire into the possible influence of soil moisture.

Since the publication of Gilman's paper, plant pathologists have continued to make observations on the influences of air and soil temperatures upon the growth of the plants and upon the occurrence of yellows in the Wisconsin cabbage fields. During the midsummer months when the soil is dry and hot, cabbage plants begin to languish and assume a pale, lifeless color. Growth is markedly checked, especially when these conditions obtain for two or three consecutive weeks. It is during this trying period that yellows develops in its most destructive form on the "cabbage sick"<sup>4</sup> soils. During very dry hot seasons even the resistant strains of cabbage, such as the Wisconsin Hollander, may show a considerable percentage of incipient disease, but upon the return of more favorable weather conditions (rain and lower temperature) they usually overcome the attack and produce marketable heads. Such field observations soon convince one that the occurrence and severity of yellows are closely correlated with the influences of soil temperature and soil moisture, and the presumption would seem to be that these influences relate both to host and to parasite.

The writer undertook to learn more exactly the importance of these factors as they affect young seedlings, beginning the work in the summer of 1917. The purposes outlined were: (1) To determine the range of soil temperature for the occurrence of yellows in cabbage seedlings; (2) to study the influence of such soil temperatures upon the normal growth of cabbage seedlings in noninfested soil; (3) to study the influence of high soil temperature upon the relative susceptibility shown by the resistant strain, that is, upon the possible "breaking down" of resistance; (4) to determine in a like manner the influence of soil moisture both upon the growth of cabbage plants and upon the occurrence of yellows in them.

<sup>1</sup> Accepted for publication Jan. 5, 1922.

<sup>2</sup> The writer wishes to make grateful acknowledgment to Prof. L. R. Jones, of the University of Wisconsin, for supervision and helpful suggestions during the progress of this work.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 86-86

<sup>4</sup> The term "sick" or "cabbage sick" soil as used in this paper indicates soil infested with *Fusarium conglutinans*.

Cabbage seedlings have been found to lend themselves fairly well, distinctly better than the larger plants, to these studies. The reasons for this are that (1) the symptoms of the disease become evident very soon after the seedlings emerge from the soil and diagnosis is easy and sure for the experienced observer; (2) the experiments can, therefore, be carried to definite conclusions within a relatively short time; (3) for such a brief period the seedlings develop well in fairly crowded plantation so that adequately large numbers can be used in pot culture; (4) the seedlings behave well under a reasonably wide experimental range of variation in soil temperature and soil moisture.

#### INFLUENCE OF TEMPERATURE UPON THE GROWTH OF *FUSARIUM CONGLUTINANS* IN CULTURE

A partial knowledge of the influence of temperature upon the growth of *Fusarium conglutinans* has already been obtained. Gilman (8) in his early work found that the conidia did not germinate within 72 hours when exposed at 8° to 10° C. in Van Tieghem cells, while only 3 hours were required for germination at 33° and 8 hours at 21°. His study of the growth of the mycelium was limited to a narrower range of temperature. The mycelium grew slowly at 8° to 10° and most vigorously at 25°, the highest temperature used in his series. An intermediate growth rate was obtained at 18° to 22°. It is seen that data on the upper limits of temperature were still lacking; therefore, it seemed advisable to determine these limits before taking up a study of the relation of soil temperature to the occurrence of yellows.

A fragment of mycelium from a young culture of *Fusarium conglutinans* was placed in the center of plates (10 by 100 mm.) of 2 per cent potato-dextrose agar, titrating + 10 Fuller's scale. The plates were then placed in incubators at a series of temperatures ranging from 7° to 37° C., those at the higher temperatures being inclosed in moist chambers to guard against inhibiting desiccation. Two plates were carried at each temperature, and measurements were made daily for seven days. The results at the end of four days and those at the end of seven days are given in Table I and are also shown graphically (fig. 1) for comparison with the percentage of yellows.

TABLE I.—Growth of *Fusarium conglutinans* at different temperatures on potato agar

Temperature.	Diameter of colony at various ages.	
	4 days.	7 days.
°C.	Cm.	Cm.
7 to 8	No growth.	Slight growth.
11 to 12	0.5	1.6
17 to 18	1.3	2.9
21 to 22	1.6	3.5
24 to 25	3.2	6.0
27	3.3	6.0
30	1.2	2.0
35	.3	.6
37	No growth.	No growth.

It is readily seen that if diameter of colony is used as a criterion, the optimum temperature for growth on potato agar plates for brief periods of time is found to be between  $24^{\circ}$  and  $27^{\circ}$  C., with a rather sudden dropping off toward the extremes. There was no growth at  $37^{\circ}$ , but the fungus was not killed at this temperature, as was shown by transferring the plates to an incubator held at  $24^{\circ}$ . After exposure for 10 days at  $24^{\circ}$  the colonies on the plates which had previously been exposed at  $8^{\circ}$  and  $37^{\circ}$ , respectively, showed vigorous growth.

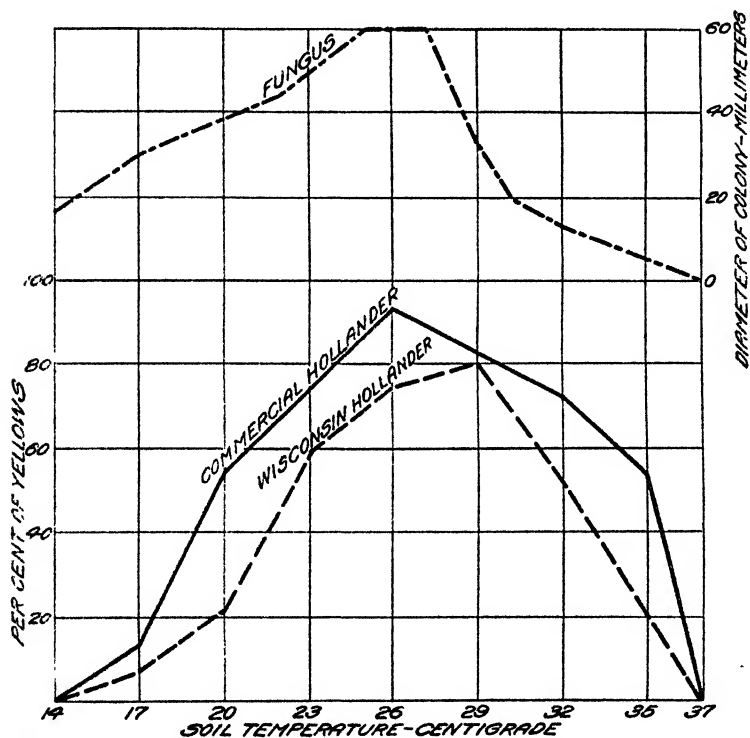


FIG. 1.—Comparison of rate of growth of *Fusarium congluticans* on agar plates with development of yellows in cabbage seedlings given in Tables I and V, plants grown 20 days from seed in naturally infested soil.

In addition to these differences in vegetative growth, the fungus exhibited a difference in sporulation at the different temperatures. At  $14^{\circ}$  C. the colonies were raised, with a leathery stroma, and produced abundant conidia. This character became less manifest up to  $24^{\circ}$ , where there was very little aerial growth and there were practically no conidia. Above  $29^{\circ}$  the colonies were again raised and produced abundant chlamydospores but no conidia. Table I shows that the growth of the fungus dropped off more suddenly above  $27^{\circ}$  than below, yet it will be seen (fig. 1) that the percentage of yellows was higher at  $35^{\circ}$  than at  $17^{\circ}$ . It is evident, therefore, that rate of growth of the fungus in culture is not directly proportional to percentage of disease at all temperatures.

Although the vegetative growth of the parasite is retarded at the higher temperatures, the data do not indicate that the pathogenicity is reduced in like proportion.

#### INFLUENCE OF SOIL TEMPERATURE UPON THE GROWTH OF CABBAGE SEEDLINGS

Inasmuch as it was presumed at the outset that soil temperature affects both host and parasite, it was considered of much importance to study separately its effect upon the cabbage plants in noninfested soil as well as upon the fungus in culture. The results of the studies of the influence of temperature upon the fungus have already been presented

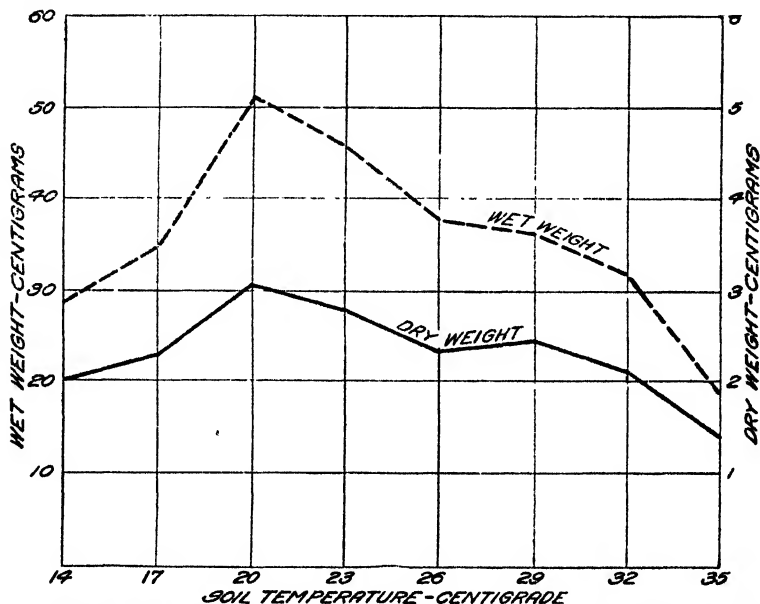


FIG. 2.—Comparison of wet weights and dry weights of shoots of healthy Wisconsin Hollander cabbage seedlings given in Table II, grown 20 days from seed during February and March

In the experiments conducted for the purpose of determining the influence of soil temperature upon the seedlings, the general appearance of the plants and the dry weight of the shoots and roots were used as bases for comparison. For preliminary data use was made of the Wisconsin Hollander plants which had been grown 20 days in sterilized soil as controls on the relation of soil temperature to the occurrence of yellows. The methods of planting and controlling the soil temperature will be explained in connection with the experiments for studying the influence of soil temperature upon the occurrences of yellows. At the end of 20 days six plants were taken for each temperature and both wet and dry weights were determined. The tops were cut off at the surface of the soil and dried in a constant-temperature oven for 18 to 24 hours at 95° C. The average results of two such series are given in Table II and shown graphically in figure 2. These first trials were conducted during February and

March when the light was weak and the day short. Other experiments were carried out in May and June when the light was stronger and the plants were grown for a longer period of time.

TABLE II.—Weights of Wisconsin Hollander seedlings grown 20 days from seed at different soil temperatures

Soil temperature.	Wet weight per plant.	Dry weight per plant.	Ratio of wet weight to dry weight.
°C.	Gm.	Gm.	
14.5	0.29	0.020	14.5
17.0	.34	.023	14.7
20.0	.51	.031	16.4
23.0	.45	.029	15.5
26.0	.37	.023	16.0
29.0	.36	.024	15.0
32.0	.32	.022	14.5
35.0	.18	.014	12.8
38.0	.06	.006	10

It will be seen from Table II that 20° C. proved to be the optimum soil temperature for the growth of these cabbage plants for the first 20 days. At soil temperatures above 20° there was a gradual decrease in weight up to 35°, where there was a sudden drop; at 38° the plants soon died. Accompanying this difference in weight was a marked difference in the appearance of the plants grown at low and at high temperatures. At 23° and below they were stocky and had a dark green color. There was also a slight difference in the height and size of the plants at the different temperatures. From 26° to 32°, inclusive, the plants became more strict; that is, the petioles were proportionately longer and approached a vertical position. The color also graded into a lighter shade of green. At 35° the plants were decidedly stunted, and they assumed a distinctly pale green color.

Moreover, if we consider the ratio of wet weight to dry weight, a marked difference will be noticed at the different soil temperatures. At the intermediate temperatures, where the greatest wet and dry weights developed, the ratio of the wet weight to dry weight was greatest. This simply means that the plants were most succulent at these temperatures, and it seems possible that for this reason they may have offered a more favorable medium for the invasion of the parasite than was offered by the plants at the extreme temperatures. This suggestion is favored to some extent by the occurrence of a higher percentage of yellows in plants growing in naturally infested soil at these temperatures. On the other hand, as will be seen later, the highest percentage of yellows occurs in plants grown in artificially inoculated soil above 26° C., where the moisture content of the plant is lower and the plants must be in an abnormal condition. These results indicate that some factor other than succulence of the host plant must be concerned in determining the degree of infection.

In order to determine whether similar temperature relations obtained for longer periods, the seedlings used as controls in the third experiment in studying the disease were allowed to grow at the different soil temperatures for 46 days before the weight determinations were made, as compared with 20 days in the former series. The roots were then washed



out of the soil and dry weights were made separately for roots and shoots. The results, consisting of the average per plant for the nine plants from each temperature, are shown in Table III. This experiment was conducted in May and June, 1919, when the air temperature ranged from 18° to 20° C., with a rise of 10° to 12° during the middle of the day.

TABLE III.—Dry weights of cabbage plants grown 46 days from seed at different soil temperatures

Soil temperature.  °C.	Dry weight per plant			
	Wisconsin Hollander.		Commercial Hollander.	
	Shoot.	Root.	Shoot.	Root.
	Gm.	Gm.	Gm.	Gm.
14 . . . . .	0.245	0.029	0.185	0.027
17 . . . . .	.316	.033	.306	.048
20 . . . . .	.281	.042	.262	.041
23 . . . . .	.272	.044	.223	.037
26 . . . . .	.254	.041	.192	.025
29 . . . . .	.302	.052	.252	.035
32 . . . . .	.236	.030	.260	.036
35 . . . . .	.207	.025	.204	.017

It may be seen from Table III that the weights are not consistent at all temperatures with those of plants 20 days old, although the temperature for optimum growth remains the same. The weight at 26° C., is less than that at 23° and 29°, thus giving a distinct bimodal curve when the weights are plotted against temperature. This condition exists alike with roots and shoots of both strains.

Inasmuch as the moisture content of the soil was not kept constant by weight, it was thought that a difference in moisture, provided there were such, might have been responsible for the irregular growth of the plants. Therefore, another experiment was conducted, during the winter of 1919, in which the whole soil mass was kept up to a uniform moisture content by weight. The soil was made up of three parts of fairly rich, virgin loam and one part of clean sand. The water-holding capacity of this soil was 39.2 per cent (in 1 cm. tube), or 28 per cent calculated on a wet-weight basis, and the moisture was kept at about 15 per cent by weighing the receptacles at the higher temperatures every day and less frequently at the lower temperatures and restoring the lost moisture. In place of the cinders used in the other experiments, a 3-inch pot was inverted in the bottom of the receptacle, a glass tube inserted in a hole in the bottom of the pot, and the soil filled in around this and tamped fairly firmly. All of the water was supplied through the tube. The seed was planted as previously described. After the plants were about 5 days old, the stand was thinned to three in each receptacle, and the soil surface was covered with mineral wool. Both the Wisconsin Hollander and the Commercial Hollander were used, the receptacles being handled in duplicate at each temperature.

The experiment was begun October 22 and concluded December 15, 1919, a total of 53 days. The air temperature during this period ranged from 14° to 18° C., with a daily rise of about 5° in the middle of the day.

The roots were washed out of the soil and dry weights made of both shoots and roots in the usual way. The results in Table IV show the average of six plants from each temperature. These results are shown graphically in figures 3 and 4.

TABLE IV.—Dry weights of cabbage plants grown 53 days from seed at different soil temperatures

Soil temperature. °C	Wisconsin Hollander		Commercial Hollander.	
	Shoot.	Root	Shoot	Root.
	Gm.	Gm.	Gm.	Gm.
14	0.230	0.034	0.267	0.039
17	.305	.036	.313	.046
20	.302	.036	.313	.040
23	.239	.019	.236	.020
26	.258	.021	.255	.019
29	.200	.020	.309	.024
32	.185	.017	.233	.012
35 <sup>a</sup>	.021	.006	.053	.005

<sup>a</sup> The temperature rose to 38° C. during two successive nights soon after the plants appeared above ground, this may account for the extremely low weights occurring at this temperature.

This table shows that 17° to 20° C. was also the optimum soil temperature for the growth of cabbage seedlings for this longer period. The dry weights obtained at some temperatures were even lower than the weights

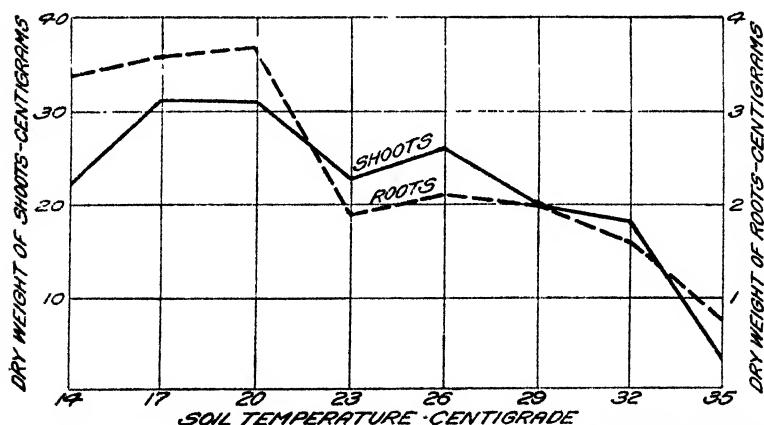


FIG 3.—Comparison of dry weights of shoots and roots of healthy Wisconsin Hollander seedlings given in Table IV, grown 53 days from seed during November and December.

obtained for plants only 46 days old, but the difference in the composition of the soil was probably sufficient to account for the difference in weight. The bimodal condition was evident in this experiment also, but in this case the lowest weight occurred at 23° instead of 26°, as in the previous experiment. The bimodal condition in weight and the difference in color were probably due to the effect of temperature upon the physiological balance of the plants, but since no analyses have been made of plants

grown at the different temperatures, it is impossible to offer any definite explanation for these differences. However, by comparing figure 1 with figures 3 and 4, it will be seen that the higher percentages of yellows occurred at and slightly above the temperature at which the drop in dry weight of the plant occurred, which was also the temperature at which the fungus grew most rapidly in culture. It is probable that both reduced vigor of the plant and optimum temperature for growth of the fungus played a part in producing a higher percentage of yellows at these temperatures.

There is a marked difference in the external characters of the root systems of the plants grown at different temperatures. Roots grown at 14°, 17°, and 20° C. are coarse, light in color, have a thick cortex, and spread uniformly throughout the soil mass. Beginning at 23° the roots

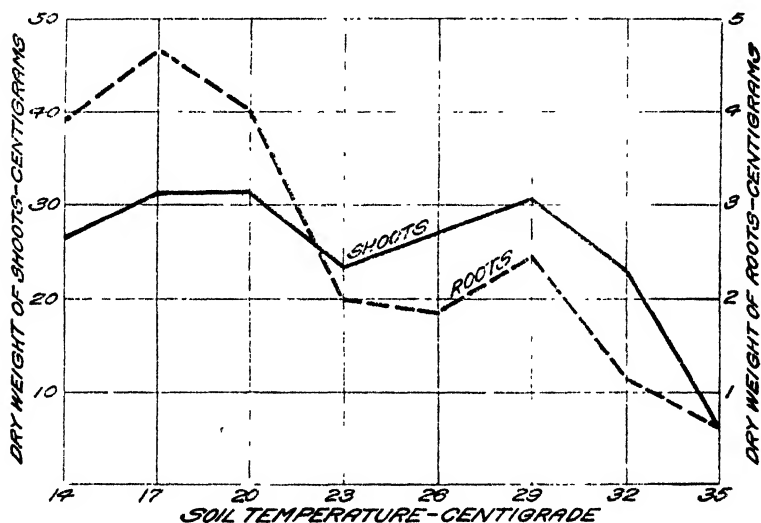


FIG. 4.—Comparison of dry weights of shoots and roots of healthy Commercial Hollander seedlings given in Table IV, grown 53 days from seed during November and December

are finer, darker in color, and shorter, until at 35° the number of roots and the extent of the system are greatly reduced and the color becomes dark brown. A comparison of the shoots and roots of plants grown at 20°, 26°, and 32° is shown in Plate 1. All of these variations suggest that fundamental differences occur in the anatomical structures of the tissues involved and possibly in their chemical composition, but analytical studies on these differences have not as yet been undertaken. It seems evident, however, that such variations in external appearance of parts of the host at different temperatures may be regarded as indices of the more important internal effects of temperature. They are probably accompanied by a difference in the rate of manufacture of carbohydrates and proteins and in the disposition of these products. In turn, these differences in assimilation and physiological balance could affect the cell walls and protoplasm in a manner which would make the plant more susceptible to the attacks of the fungus.

# INFLUENCE OF SOIL TEMPERATURE UPON THE OCCURRENCE OF THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS

## GREENHOUSE EXPERIMENTS

**METHODS AND APPARATUS.**—The apparatus employed for controlling the soil temperature in these experiments was what is termed the Wisconsin soil-temperature tank. Since it differs only in detail from that used by Johnson and Hartman (9) and described by Jones (10), further description is considered unnecessary in this connection. A series of nine separate tanks or compartments was employed, with extreme temperatures of 14° and 38° C. and a difference of 3° between successive tanks, thus giving the graduated series 14°, 17°, 20°, 23°, 26°, 29°, 32°, 35°, and 38°. The temperatures below 23° were regulated by running in a small amount of cold water two or three times a day as the case required, while the temperatures from 23° to 38° inclusive were maintained through the use of carbon electric lamps under thermostatic control. During the earlier experiments some of the temperatures were regulated through the use of steam, but this method was abandoned when the electric lamps were installed. With personal attention two or three times every 24 hours, the temperatures were kept fairly constant, rarely varying more than 1° from that desired.

The air temperature in the tank room was kept constant within a range of a few degrees. During the course of the first experiments it ranged from 18° to 22° C. with a maximum rise of 5° to 10° for one or two hours on bright days. During the spring of 1919 the air temperature range was from 14° to 18° with a similar rise at midday on bright days.

The surface of the soil in the receptacles was covered with a half-inch layer of mineral wool to insulate it from the air temperature and to reduce evaporation. Even with this protection, the surface inch of soil at the higher temperatures usually registered from 1° to 1½° lower than the water in the tanks. However, the deeper layers of soil registered the same temperature as the water, and the plant roots were found to be distributed largely in the layers below the first inch. Therefore the temperatures recorded for the different series are those at which the water was maintained in the tanks in which the receptacles were suspended.

**RECEPTACLES.**—The receptacles or culture pots were made of galvanized sheet iron, cylindrical, 6 inches in diameter and 10 inches deep. They were made ready for use by placing a layer of fine coal cinders about 2 inches deep on the bottom with soil on top of this up to within 1½ inches of the top. All of the water was supplied through a glass tube 12 mm. in diameter which was placed in the center of the receptacle of soil with the lower end inserted in the cinders. After the receptacles were filled with soil, they were so placed in the tanks that the surface of the soil was level with the surface of the water in the tank. They were then allowed to remain in the tanks one or two days before the seed was planted.

**SOIL.**—The naturally infested soil used in all the experiments was obtained from a uniformly "cabbage sick" field in Kenosha County, Wis. It was a dark clay loam containing some gravel. Before being used the soil was screened and uniformly mixed with coarse sand in the ratio of five parts of soil to one of sand. After the sand was added, the portion to be used as control was autoclaved at 5 pounds pressure for two hours and then allowed to stand for one week before it was used.

**HISTORY OF SEED AND METHODS OF PLANTING.**—The seed used in all the experiments was from the same lots of the Late Wisconsin Hollander and Commercial Hollander strains. The Late Wisconsin Hollander was the strain of Hollander which has proved to be highly resistant to the *Fusarium* disease under field conditions, as recently described by Jones, Walker, and Tisdale (12). This seed was grown in 1917 by S. B. Walker at Racine, Wis. The seed of the commercial strain was obtained from the L. L. Olds Seed Co., Madison, Wis. No information could be had as to where and when it was grown, but it was considered a good representative of the Commercial Hollander type. It had been proved to be about 99 per cent susceptible when grown in "sick" soil.

In the first and third tests, three receptacles in each tank were planted with Wisconsin Hollander and three with the Commercial Hollander. Two of the three receptacles in each set contained "sick" soil and one contained sterilized soil. In the second experiment only two receptacles were used for a strain in each tank, one containing "sick" and the other sterilized soil. When the plants were well above ground, the stand was thinned to 10 plants in each receptacle, except in certain cases, and the surface of the soil was covered with a half-inch layer of mineral wool.

**THE EXPERIMENTS.**—Four separate tests were conducted for measuring the influence of soil temperature upon the occurrence of yellows in cabbage seedlings, three with naturally infested soil and one with artificially inoculated soil. Three of the tests were conducted during the winter of 1917 and the spring of 1918, and one in the spring of 1919. In all three experiments with naturally infested soil the behavior of the host plant was similar, and the percentage of disease checked fairly closely. Of course, the intensity of light was greater in the spring than during the winter, but it was reduced in the spring by nailing cheesecloth on the inside of the roof of the greenhouse above the tanks. The final data were recorded 20 days after the seed was planted; they show the percentage of yellows and the percentage of plants dead by this time. This length of period was chosen because in the first experiment all of the plants of the commercial strain at 32° C. had developed yellows within 20 days.

**METHODS OF EXPRESSING DATA.**—The data in the greenhouse experiments were taken daily after the disease began to develop. In the first four experiments the task of taking data consisted merely in recording the number of diseased plants and the number which died as a result of the disease. The percentage of yellows includes the plants which showed the disease in incipient stages as well as those which died from the disease. Except in doubtful cases diagnoses of yellowed plants were made from the external symptoms in the leaves; the doubtful cases were cultured on agar plates.

**EXPERIMENTAL DATA.**—The data given in Table V and figures 1 and 5 show that *Fusarium conglutinans* is capable of producing yellows in both the susceptible Commercial Hollander and the resistant Wisconsin Hollander seedlings over a wide range of temperature, the minimum being 17° and the maximum about 35° C. At the lower temperatures, however, the disease developed more slowly and less destructively than at higher temperatures (fig. 6). The Wisconsin Hollander strain was even less severely attacked at the lower temperatures than the commercial strain. Plate 2, A-C, shows a contrast of the two strains growing in "sick" soil at 17°. At 15° the disease did not develop even in the most susceptible strains. The optimum soil temperature for the occurrence of yellows in the commercial strain in naturally infested soil

was about  $26^{\circ}$  and in the Wisconsin Hollander about  $29^{\circ}$ . These temperatures practically coincide with that for growth of the causal organism on potato agar, but are several degrees higher than the optimum for growth of the seedlings. These results differ from those reported by Tisdale (13) for flaxwilt and those reported by Clayton (5) for tomato wilt only in that the optimum temperature for growth of the cabbage plant is lower than that for development of the disease and growth of the parasite. The same temperature relations do not obtain for other types of diseases attacking the underground parts of plants. Balls (1, 2) reported that the soreshin disease of cotton developed most destructively at  $17^{\circ}$  to  $23^{\circ}$  C., while the optimum for the growth of host and parasite was about  $28^{\circ}$ . Johnson and Hartman (9) reported quite similar relations for the rootrot of tobacco.

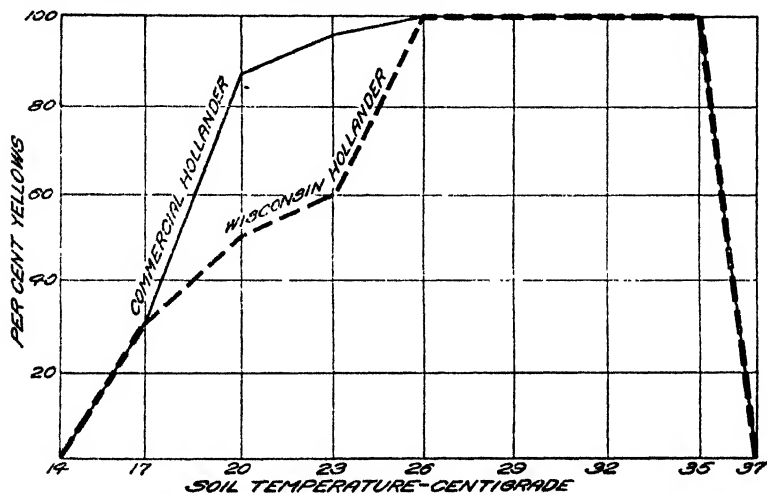


FIG. 5.—Comparison of development of yellows in Commercial Hollander and Wisconsin Hollander seedlings given in Table V, grown 20 days from seed in artificially inoculated soil.

Although the percentage of yellows in the resistant Wisconsin Hollander was rather high during the early period of growth, it was lower at all temperatures than that of the susceptible commercial strain. The greatest differences in percentage of yellows between the two strains occurred at  $20^{\circ}$  and at  $35^{\circ}$  C., where they amounted to about 35 per cent. In artificially inoculated soil at  $26^{\circ}$  and above the two strains seemed to be equally susceptible (fig. 5). The cause for such a wide difference between the pathogenicity of *Fusarium conglutinans* in naturally and in artificially inoculated soil has not been determined. It is apparent, however, that this difference is in part due to the direct relation of the higher temperatures to the stimulation of the *Fusarium*. This relation becomes especially evident when the fungus is growing in pure culture free from the complicating relations with the normally associated soil flora.

Other workers have obtained similar results with vascular parasites. Edgerton (6, 7) found that a much higher percentage of *Fusarium*-wilt of tomato developed in the sterilized, reinoculated soil than in the unsteril-

ized soil, but at the same time the resistant tomato was very resistant in the seedling stage. He did not state the temperature at which the plants were grown, however, and temperature is a potent factor in the development of cabbage yellows. At 23° to 32° C. seedlings of the resistant strain of cabbage show a high percentage of yellows, but the plants which escape the disease usually remain healthy. W. H. Tisdale (14) found the resistant strain of flax to be highly resistant even in the seedling stage.

The upper limit for the growth of *Fusarium conglutinans* in pure culture is about 35° C., yet it produced 100 per cent yellows in plants grown at this temperature in artificially inoculated soil. As previously stated, the temperature of the first inch of soil at 35° registers from 1° to 1½° lower than the water in the tank, and it may be that the fungus made its successful attack upon these roots in the first inch of soil.

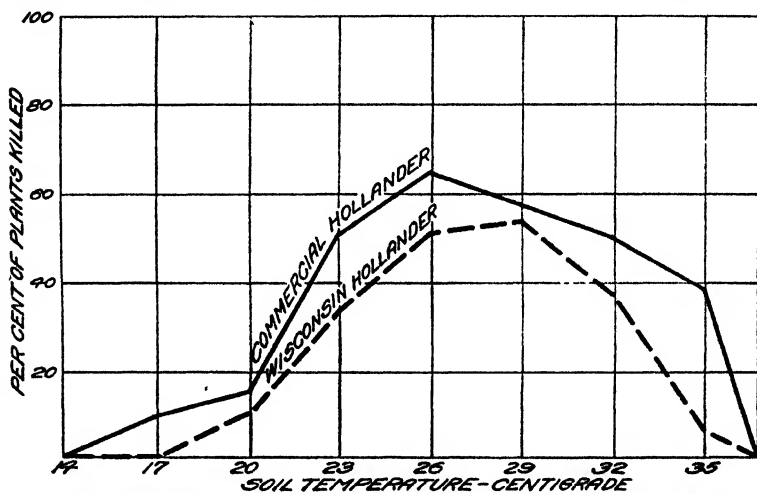


FIG. 6.—Comparison of death rate from yellows in Commercial Hollander and Wisconsin Hollander seedlings given in Table V, grown 20 days from seed in naturally infested soil.

The data presented in Table V and figure 6, especially those for artificially inoculated soil, show that the highest percentage of yellows occurred at temperatures above that most favorable for the seedling. This indicates that resistance is probably broken down at these higher temperatures. However, the chief difficulty in determining to what extent the increase in percentage of yellows at higher temperatures is due to "breaking down" of resistance is the fact that the nature of the resistant character has not been analyzed. Until its true nature has been determined, it will be impracticable to learn by the common method of testing for resistance in plants, that is, by subjecting both host and parasite to the different temperatures, just how much temperature affects the resistant character. However, the results obtained with artificially inoculated soil indicate that either the host becomes less resistant or the fungus more pathogenic at the higher temperatures. The latter condition was not in evidence with susceptible plants, but the decided stunting of the plants would seem to indicate that they may be less resistant.

TABLE V.—Percentage of cabbage seedlings showing yellows at different soil temperatures after growing 20 days from seed

[illegible]

The death of the plants at this temperature ( $38^{\circ}$ ) was evidently due to the effect of high temperature alone. No culture of *Fusarium* was ever obtained from the plants.



## INCUBATION PERIOD OF THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS AT DIFFERENT TEMPERATURES

In all of the experiments reported in this paper a great difference in the incubation period of the disease was observed in the plants grown at the different soil temperatures, and also to some degree between plants in the same receptacle. The incubation period, as spoken of in this connection, was reckoned from the time the seed was planted rather than from the date the plants emerged from the soil, because at temperatures below 23° C. two or three days more were required for the plants to emerge from the soil than at the higher temperatures, although the seed coats ruptured practically as soon at the lower temperatures. Because of this fairly uniform breaking of the seed coats, the seedlings at the different temperatures were exposed to the fungus for about the same length of time.

In every case the disease appeared first at 26° to 32° and last at 17° C., with a gradation between these extremes. The incubation periods at these temperatures were 7 days and 18 days, respectively. At 26° to 32° the disease appeared in some plants within 1 or 2 days after the plants emerged from the soil, while others in the same receptacle appeared perfectly normal and healthy for several days longer. At the lower temperatures the disease was much longer in becoming manifest but at the same time showed a similar variation in length of incubation among individuals. The variation among plants grown in artificially inoculated soil was not as great as that among plants grown in naturally infested soil. A comparison of the results in the two cases is shown in Tables VI and VII. Gilman (8, p. 43, Table VI) also showed that, although inoculations were made with parts of the same culture on plants from the same pot and under conditions as nearly identical as possible, the incubation period varied widely. The cause of this variation has not been definitely determined, but the results so far obtained indicate that it is a difference in the genetic composition of the individuals. Under the conditions of these experiments the plants must have been exposed to essentially like opportunities for attack by the fungus.

When the plants were grown in "sick" soil for 20 days at 14° and then transferred to 26° C., the incubation period was only 4 to 6 days, although a smaller percentage of disease developed in the resistant seedlings than when the plants were grown from seed in "sick" soil at 26°. This short incubation period is very probably due to the fact that the root hairs were in close contact with the fungus mycelium at the low temperature, and when subjected to a more favorable temperature the fungus immediately began invasion of the roots through the root hairs. The Wisconsin Hollander plants treated in this manner showed the disease mostly in incipient form, whereas the susceptible commercial strain was as susceptible as in the earlier stages of development.

When the plants were grown for 30 to 36 days in noninfested soil and then transplanted to "sick" soil the incubation period was longer in all cases than when the seed was planted in "sick" soil. Under these conditions the incubation period was shortest at 26° C. and longest at 17°, being 11 and 17 days, respectively. It is probable that this longer period was due to the inability of the fungus to make a successful attack until new root hairs developed on the roots.

## A COMPARISON OF SUSCEPTIBILITY IN THE WISCONSIN HOLLANDER AND COMMERCIAL HOLLANDER SEEDLINGS

In all of the experiments conducted under controlled temperature and moisture conditions variations in individual susceptibility among plants have been very noticeable in both susceptible and resistant strains. Data were obtained from experiments previously reported by recording each day the percentage of plants in each can showing the disease at the different temperatures. These data are shown for both naturally and artificially infested soils in Tables VI and VII.

TABLE VI.—Variations in susceptibility among cabbage seedlings grown in naturally infested soil at different soil temperatures

Soil temperature. °C	Total number of plants	Percentage of diseased plants in each receptacle at the end of different periods after planting.									
		Commercial Hollander.					Wisconsin Hollander.				
		10 days.	12 days.	15 days.	18 days.	20 days.	10 days.	12 days.	15 days.	18 days.	20 days.
17	10	0	0	0	10	10	0	0	0	10	10
20	10	0	0	0	10	20	0	0	0	10	20
23	10	0	30	30	40	50	0	20	30	40	50
26	10	10	50	60	70	100	20	80	80	80	80
29	10	30	60	60	70	90	10	20	20	30	50
32	10	20	20	30	50	60	10	20	30	50	60
35	10	10	40	50	70	70	0	0	0	20	20
38	7	0	0	0	0	0	0	0	0	0	0

TABLE VII.—Variations in susceptibility among cabbage seedlings grown in artificially inoculated soil at different soil temperatures

Soil temperature. °C.	Total number of plants	Percentage of diseased plants in each receptacle at end of different periods after planting.									
		Commercial Hollander.					Wisconsin Hollander.				
		10 days.	12 days.	15 days.	18 days.	20 days.	10 days.	12 days.	15 days.	18 days.	20 days.
17	13	0	0	0	0	31	10	10	10	30	30
20	16	0	0	0	69	88	10	0	0	20	50
23	24	0	21	44	92	96	10	0	10	30	60
26	18	6	22	67	94	100	10	40	80	100	100
29	18	6	28	89	94	100	10	80	90	90	100
32	16	50	75	100	100	100	10	80	90	100	100
35	23	26	52	87	91	100	10	60	100	100	100
37	11	0	0	0	0	0	10	0	0	0	0

Examination of the Tables V-VII shows considerable variation in susceptibility as between individuals at all temperatures. This variation is manifest not only by length of incubation period but also by extent of infection and rate of development of the disease once infection has occurred. At and above 26° C. the incubation period is materially shortened for a great number of individual plants, especially those of the Wisconsin Hollander strain. In naturally infested soil Wisconsin Hollander plants which escape the disease for the first 20 days usually remain healthy, even though they are growing at high temperature. In artificially inoculated soil all of the plants become diseased within the first 20 days as a rule.

Plants of the Wisconsin Hollander strain which showed symptoms of yellows even when very young have been observed to overcome the attack and resume vigorous growth. Their ability to do this was less pronounced in plants grown in artificially inoculated soil. It will be seen from Table VII that a high percentage of plants were killed in the artificially inoculated soil. The sudden death of some plants, the ability of others to overcome the attack, and the ability of still others to escape the disease entirely, furnish evidence of a variation in degree of resistance of the individual plants during the early stage of development. The same condition has been known for several years to exist among varieties. However, still more significant is the fact that such variations in degree of resistance continue to appear as between individuals of the most resistant and most stabilized of the Wisconsin strains.

Our main interest lies, therefore, in the variations in resistance as between individual plants. The facts cited as bearing on this variation indicate that *Fusarium* resistance in cabbage is due to hereditary factors, probably multiple, and that many of the plants are entirely lacking in one or more of the factors for resistance or possess them in a heterozygous condition. The Wisconsin Hollander strain was selected to resist the *Fusarium* disease under certain conditions to which the plants are normally subjected in the field. Plants which resist under these conditions may not possess sufficient factors for resistance to enable them to resist under more severe conditions, as evidenced during very hot summers. Because of the fact that the seed plants are grown under conditions which permit free intercrossing, the tendency is for them to remain in this heterozygous condition even when grown in "sick" soil. When the seed plants are grown on "sick" soil, however, the plants which possess an insufficient number of factors for resistance develop the disease and are discarded. Thus, by selecting seed plants from "sick" soil, it is possible to maintain a resistant strain, under the conditions selected, even though it may not be homozygous for resistance.

#### RELATION OF AGE OF SEEDLINGS TO EXPRESSION OF THE RESISTANT CHARACTER

It has been shown (Table V) that during the first 20 days of development the Wisconsin Hollander plants are practically as susceptible to yellows at the higher temperatures as the susceptible commercial strain. On the other hand, the Wisconsin Hollander strain shows a high degree of resistance by the time the plants are old enough to be transplanted into the field. Since this condition has manifested itself for several seasons and since the plants are usually grown in soil with a fairly low tem-

perature before they are transplanted, there appear to be only two possible explanations for the low degree of resistance in the young seedlings, namely: (1) the resistant character is not fully expressed in the young seedlings and becomes more manifest as the plants grow older, or (2) the high temperatures inhibit or modify the expression of the resistant character in seedlings. Experiments have furnished some evidence favorable to both hypotheses. Data supporting the second have already been presented and discussed.

The initial experiments for testing the first theory were conducted by transferring the receptacles to the 26° C. tank after the plants had grown 20 days in infested soil at 14°. Plants of the commercial strain grown under similar conditions were transferred at the same time for controls. The air temperature was the same as that recorded for other experiments conducted at the same time, 14° to 18°. In Table VIII the results obtained at the end of 10 days are compared with the results obtained with seedlings which had been growing at 26° continuously for 20 days.

Because the number of plants used in these tests was small, the infection of one or two more plants in one test than in another made a considerable difference in the percentage of yellows. Even so, the results are consistent enough to indicate that the Wisconsin Hollander plants are more resistant after 20 days than when younger. In Table VIII it may be seen that there is an average difference of 54 per cent of yellows between the older plants of the Wisconsin Hollander and the Commercial Hollander strains at 26°C., while with the younger plants there is a difference of only about 14 per cent. At the same temperature the difference in the percentage of plants killed is equally striking. With the younger plants there is a difference of only 18 per cent, while with the older ones it amounts to 67 per cent. The disease developed very slowly in the Wisconsin Hollander seedlings, and at the conclusion of the experiment most of the affected plants showed only slight symptoms of yellows, while the reverse condition was manifest in plants of the commercial strain.

These investigations were carried further by planting both kinds of seed in sterilized soil and later transplanting the seedlings to "sick" soil. The plants for the first experiment were grown for 30 days in sterilized soil in flats. The temperature of both air and soil ranged from 18° to 22°C. during this period. In the first experiment, which was conducted during May and June, 1918, only three different soil temperatures were used in the series, but the complete series of temperatures from 14° to 35° was employed for the second. Three plants were set in each receptacle, and after transplantation on May 10 the receptacles were exposed to room temperature (18° to 22°) for three days in order to give the plants an equal chance to recover from the shock of being transplanted. The surface of the soil was covered with mineral wool and the receptacles were placed in the tanks at 15°, 17°, and 26°. The final results, recorded 30 days later, are given in Table IX. The air temperature during this experiment ranged from 18° to 22°, with a sudden rise of 10° to 15° for a few hours during the middle of the day. A contrast of the susceptible plants grown in "sick" soil at 15° and at 17° is shown in Plate 2, D, E.

TABLE VIII.—Influence of age of cabbage seedlings upon the occurrence of yellows

Date planted	Condition of soil	Wisconsin Hollander.						Commercial Hollander.					
		Grown in "sick" soil 20 days from seed, at 26° C.			Grown in "sick" soil 20 days at 14° C., then transferred to 26° for 10 days.			Grown in "sick" soil 20 days from seed, at 26° C.			Grown in "sick" soil 20 days at 14° C., then transferred to 26° for 10 days.		
		Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.
Feb. 1, 1918 .....	Naturally infested. ....	20	80	50	10	50	0	19	78	56	9	89	44
Mar. 29, 1918. ....	do .....	10	70	20	10	30	10	10	100	50	10	100	100
Average results of two experiments.	Naturally infested soil ..	30	75	35	20	40	5	29	89	53	19	94	73
Mar. 29, 1918. ....	Artificially inoculated...	10	100	100	10	50	10	10	100	100	10	100	100
May 5, 1919. ....	do .....							18	100	100	18	100	100

TABLE IX.—Number of cabbage plants which showed yellows at the end of 30 days after transplantation to "sick soil"

Temperature.	Number of plants.	Wisconsin Hollander			Commercial Hollander		
		Number healthy	Number yellow.	Number dead.	Number healthy	Number yellow.	Number dead.
°C.							
14	3	3	0	0	3	0	0
17	3	3	0	0	0	3	2
26	3	3	0	0	0	3	3

All of the Wisconsin Hollander plants remained healthy throughout this experiment, whereas all of the susceptible commercial plants at 17° and 26° C. developed typical yellows symptoms. At 26° yellows began to appear after 11 days and at 17° after 17 days. All three plants at 26° were dead after 17 days.

In the second experiment, plants were grown in sterilized soil 36 days from seed before they were transplanted into "sick" soil on May 24, 1919. The air temperature ranged from 22° to 25° C. during this period, but the subsequent treatment was similar to that in the preliminary experiment. The final results, recorded on June 13, are shown in Table X.

TABLE X.—Number of cabbage plants which showed yellows at the end of 20 days after transplantation to "sick" soil

Temperature.	Wisconsin Hollander.			Commercial Hollander.		
	Number healthy.	Number yellow.	Number dead.	Number healthy.	Number yellow.	Number dead.
° C.						
14	3	0	0	3	0	0
17	3	0	0	2	1	0
20	3	0	0	0	3	0
23	2	a 1	0	0	3	2
26	1	a 2	0	0	3	3
29	1	a 2	0	0	3	3
32	2	1	0	1	2	0
35	3	0	0	0	3	0

a The Wisconsin Hollander plants showed yellows in none but the lower leaves, and there only in incipient form.

Here again, all three of the Commercial Hollander plants developed typical yellows at 23° to 29°, inclusive, whereas only one or two of the Wisconsin Hollander plants showed any symptoms of the disease at all and then only in incipient form in the lower leaves. The number of plants is too small to warrant definite conclusions, but the evidence from both field and greenhouse experiments seems sufficient to indicate that older plants are more resistant than younger ones, and also that resistance becomes more stable as the plants grow older. There is also some indication here that resistance was weakened by starting the plants at a higher temperature, but the experiment needs to be repeated with larger numbers of plants before final conclusions on this point are justified.

The fact that a higher percentage of yellows develops at temperatures unfavorable for the growth of the seedlings is further evidence that the resistance is "broken down" at these temperatures. It has been reported that hereditary characters in several different organisms have been modified by changing the environment (including temperature) of the individuals. Baur (3, p. 8-9) found that flower color, an inherited character of the Chinese primrose (*Primula sinensis rubra*), was conditioned upon temperature. The plants were grown by the usual method until about one week before the blossoming stage; then some of them were put into a warm room (30° to 35° C.) and the others into a cool room (16° to 20°). The plants at the higher temperature produced pure white flowers, while those at the lower temperature produced flowers of the normal red color of the variety. Biffen (4), in his work on the inheritance of resistance to yellow rust in wheat, concluded that any factor altering in any way the metabolic processes of the plant in turn alters the degree to which it is attacked by yellow rust and probably other fungi as well. He also found that a variety of wheat which under ordinary conditions of cultivation would be classed as moderately susceptible may be severely attacked when large amounts of nitrates are added to the soil.

## FIELD EXPERIMENTS

### FIELD OBSERVATIONS

Jones and Gilman (11) first recorded the fact that severe attacks of cabbage yellows are associated with hot, dry weather. Gilman (8) analyzed this evidence in detail and showed that the incubation period of the disease under field conditions is also materially influenced by soil temperature. The incubation period was about 14 days in 1912 when the mean daily temperature 6 inches below the surface was about 23° C. and 20 days in 1914 when the mean daily temperature at the same depth was about 20°.

Similar observations were made during the summers of 1917 and 1919 in the same field by the writer. In 1919 the mean daily soil temperature 4 inches below the surface was 25° C. at the time of transplantation. Thirteen days later 30 per cent of the Commercial Hollander plants showed typical yellows. This high percentage of diseased plants, together with the different stages of symptoms at that time, indicated that the disease had been present in some of the plants at an earlier date. According to these observations, the incubation period of the disease may vary under field conditions from about 12 days with a

mean soil temperature of about 25° to 20 days with a mean soil temperature of about 20°. It has also been repeatedly observed (11, 8) that the plants which escape or survive the disease for the first month may remain healthy during the remainder of the season.

These observations gave sufficient evidence to prove that soil temperature is an important factor in the development of yellows in cabbage plants transplanted into "sick" fields. However, they do not satisfactorily show what results might be obtained with younger seedlings planted at intervals throughout the season. Data upon experiments of this nature were considered of distinct value for comparison with results obtained in the greenhouse at different temperatures. Therefore, experiments were begun in 1917 for obtaining such data. Both the resistant Wisconsin Hollander and the susceptible Commercial Hollander were used. By beginning to plant early in the season and continuing until late in the summer, it was possible to have each successive crop of seedlings exposed to a different range of soil temperature. It was the original plan to plant the seed at intervals of 7 to 14 days and record the final data 20 days later, as was done in the greenhouse, but for various reasons this schedule could not at all times be very closely adhered to. Consequently, the data obtained in these experiments are not altogether comparable with those obtained in the greenhouse. Even so, as will be shown later, the two sets of data coincide closely enough to justify the conclusion that under field conditions soil temperature is one of the chief limiting factors in the development of yellows in young seedlings as well as in the older plants after transplantation.

#### EXPERIMENTAL METHODS

The soil temperature was recorded in 1917 in the experimental plot at 1 inch below the surface throughout the growing season and at 4 inches for a part of the season. In 1919 the temperature was recorded only at 4 inches below the surface. Fries kerosene bulb thermographs were used for this purpose. The instruments were checked once a week against a standardized thermometer to insure accuracy.

The seed was sown in short rows and observations were made every few days for a period of 20 to 30 days. In a few cases more than 30 days intervened between observations. During the driest part of the season it was necessary to water the soil at the time the seed was planted in order to insure germination. The seedlings were counted soon after they emerged from the soil, and this number was used as a basis for calculating the percentage of disease. The results of the experiments of 1917 and 1919 are given in summarized form in Table XI; the percentage of yellows and the range of the soil temperature are shown graphically in figures 7 and 8. In these figures is shown the correlation between the percentage of yellows and the curves which represent the range of mean daily soil temperatures. The mean is an average of the temperature readings taken at 2-hour intervals during the day. This is only a relative temperature, of course, because it gives no notion of the duration of the extremes, which is undoubtedly an important factor with diseases of this type.

**EXPERIMENTAL DATA.**—An inspection of Table XI and figures 7 and 8 shows that seedlings started early in the season may remain absolutely free of the yellows disease for the first 30 days, while the successive later plantings, up to about August 15, show a progressive increase in

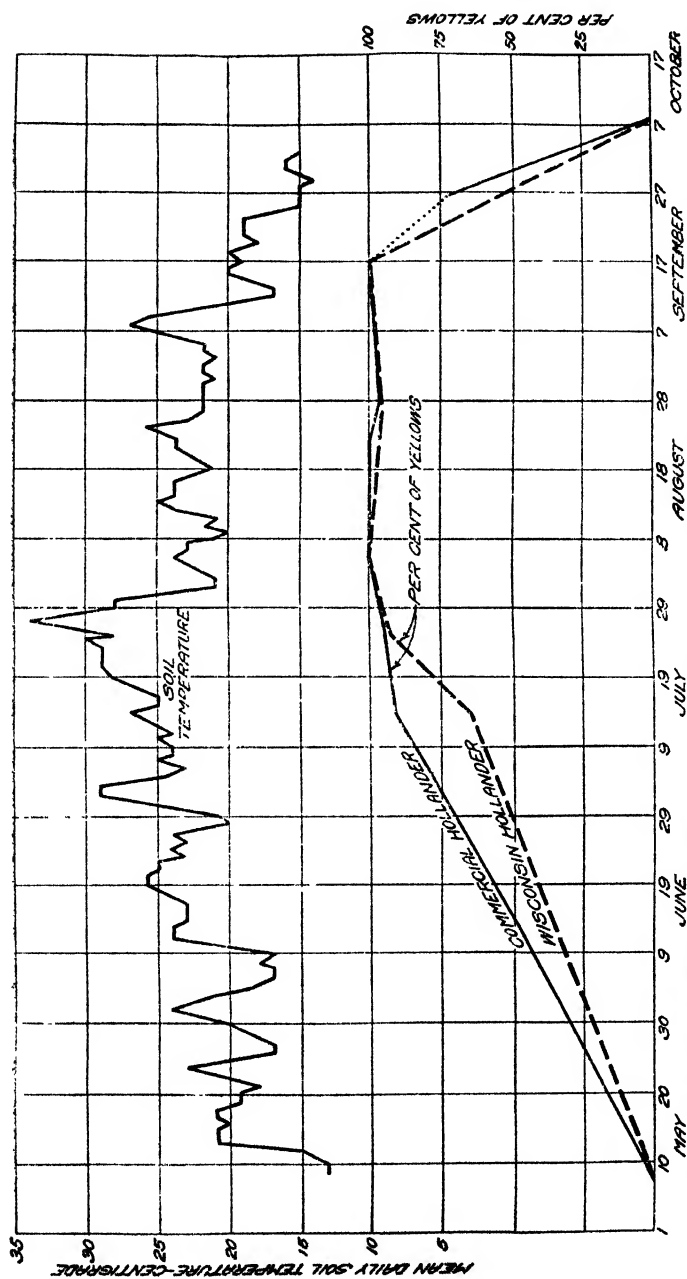


FIG. 1.—Correlation of the development of yellows in Commercial Hollander seedlings in the field, 1917, with the mean daily soil temperature 1 inch below the soil surface. Data given in Table XI



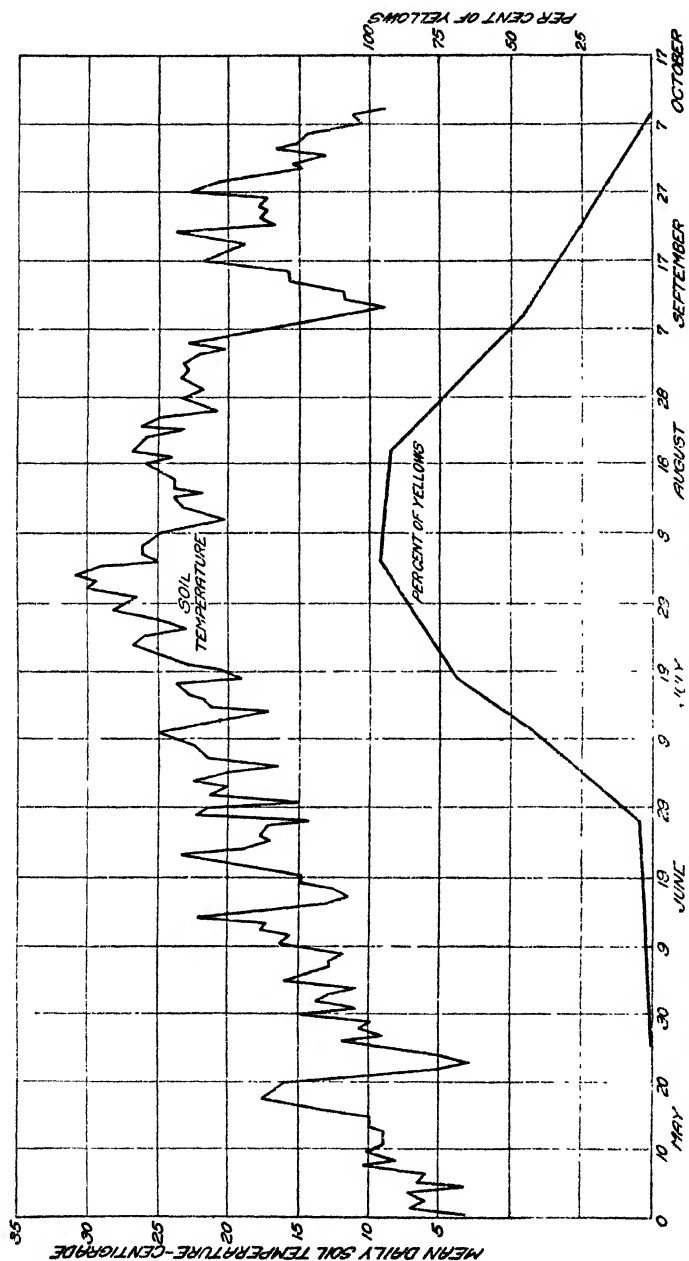


FIG. 8.—Correlation of the development of yellows in Commercial Hollander and in Wisconsin Hollander seedlings in the field, 1919, with the mean daily soil temperature 4 inches below the soil surface. Data given in Table XI.

the percentage of disease. Plantings made after the latter date showed a rapid decrease in percentage of disease until about September 15, after which the disease did not develop. It may also be observed (fig. 7 and 8) that this rise and fall in the percentage of disease correlates in general with the soil-temperature curve. The disease did not appear in the early part of the season until the soil temperature had remained above 17° C. for several days, and it ceased to develop in the latter part of the summer as soon as the temperature dropped below this point. By comparing the temperature curves in figures 7 and 8, it may be observed that the soil did not warm up as early in 1917 as in 1919 and that the development of yellows was deferred in a corresponding manner.

During the hot, dry period, which included the greater part of July and the first half of August, the disease developed as quickly as, and even more destructively than, at the high temperatures in the greenhouse. This result was probably due to the combined influences of high soil temperature and low soil moisture, each of which was found to favor rapid development of the disease under greenhouse conditions. On the other hand, the disease was found to develop more slowly in the field at the lower temperatures than in the greenhouse at constant similarly low temperatures. This difference was probably due to the effect of the wide daily fluctuation of the soil temperature upon the fungus under field conditions—that is, the short period of higher temperature might have been insufficient for the fungus to recover from the effect of the longer duration of low temperature. Often when the mean temperature was about 20° C. the range was probably below 20° all night and a large portion of the day, while it remained above the mean only a few hours.

In several instances, during both 1917 and 1919, the plants were left in the beds for the greater part of the season and observations were made upon them from time to time. By this method it was learned that most of the Wisconsin Hollander plants which were healthy at the end of the first 30 days remained so throughout the experiment. The number of plants thus escaping the disease was greater when they were started in May than when started in July or in August when the soil was hot and dry. The Wisconsin Hollander plants which were started on May 15, 1917, showed no yellows at the end of the first 33 days, and only 15 per cent 62 days after planting. There was no increase in percentage of disease between July 18 and July 26. Conversely, the plants which were started on July 14, 1917, showed 96 per cent of yellows on August 4, 21 days after planting. Sixteen days later the disease had increased to 98 per cent. These data confirm the results obtained in the greenhouse, that plants which were permitted to establish themselves before being exposed to the *Fusarium* at a temperature favorable for its attack were more resistant than plants started in "sick" soil at the optimum temperature for the fungus. In other words, the plants became more resistant with age.

TABLE XI.—Percentage of plants developing yellows when planted in the field on different dates during the season

Date of planting.	Date of observation.	Number of days between time of planting and final observation.	Total number of plants.		Percentage of yellows.	
			Wisconsin Hollander.	Commercial Hollander.	Wisconsin Hollander.	Commercial Hollander.
1917.						
May 25.....	June 27	33	100	100	0	5
June 21.....	July 11	20	100	100	15	57
June 27.....	July 18	21	100	100	22	70
July 14.....	Aug. 4	21	140	104	96	97
July 26.....	Aug. 20	25	89	205	94	94
Aug. 18.....	Sept. 8	21	400	120	48	48
Sept. 22.....	Oct. 8	16	100	100	0	0
1919.						
Apr. 6.....	May 8	32	200	200	0	0
May 8.....	June 12	35	200	200	35	45
June 18.....	July 14	26	256	173	64	92
June 30.....	July 25	25	191	96	94	96
July 14.....	Aug. 6	23	111	93	100	100
July 30.....	Aug. 22	23	269	140	97	100
Aug. 6.....	Aug. 28	22	210	266	96	97
Aug. 12.....	Sept. 16	35	390	360	100	100
Aug. 20.....	Sept. 16	26	28	131	100	100
Sept. 7.....	Sept. 27	20	152	134	53	70
Sept. 16.....	Oct. 8	22	213	280	0	0

## INFLUENCE OF SOIL MOISTURE UPON THE OCCURRENCE OF YELLOWS IN CABBAGE SEEDLINGS

## EXPERIMENTAL METHODS

The experiments for measuring the influence of soil moisture upon the occurrence of yellows were conducted during the winter of 1918 and the spring of 1919 simultaneously with, and under the same atmospheric conditions as, the soil-temperature experiments. The same kind of soil and receptacles were used, but no cinders were placed on the bottom of the receptacles. The moisture-holding capacity of the soil was found by means of 5 by 20 cm. tubes to be 46 per cent. When based upon wet weight, this gave approximately 31 per cent moisture.

The moisture content of the soil was kept constant during the experiments through the use of one Livingston cylindrical auto-irrigator, 5 by 15 cm., in each receptacle. The cup was imbedded vertically in the soil, and the water reservoirs were so placed that the moisture content became adjusted at 14.5, 19, 23, and 26 per cent, respectively, in the four sets of duplicate receptacles. The receptacles with 14.5 per cent moisture were kept in the series for only about two weeks when the water columns broke and they were not restored in time to keep them in the series. Moisture determinations were made just before the seed was planted and at the conclusion of the experiment. The percentage of moisture at the conclusion was only a few tenths of 1 per cent lower than at the beginning. The soil temperature was kept constant at 22° to 23° C. throughout the experiment.

After the soil moisture had become constant, seed of the Commercial Hollander strain was planted in the usual manner, and the final data were recorded 28 days later. A few days after the plants emerged from the soil, they were thinned to the desired stand and a half-inch layer of mineral wool was placed over the surface of the soil to equalize surface temperatures. In Table XII the average results from the duplicate receptacles are shown separately for each experiment at the end of different periods of time. The average results of the two experiments are given in Table XIII and shown graphically in figures 9 and 10.

TABLE XII.—*Influence of soil moisture upon the development of yellows in cabbage seedlings, shown at end of different periods after seeding*

FIRST EXPERIMENT, BEGUN FEBRUARY 28 AND CONCLUDED MARCH 27

Number of days after planting	19 per cent moisture.			23 per cent moisture.			26 per cent moisture.		
	Total number of plants	Percentage yellow	Percentage dead.	Total number of plants	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.
10. ....	20	0	0	20	0	0	20	0	0
12. ....		20	0		0	0		3	0
15. ....		50	0		5	0		20	0
17. ....		85	30		45	0		35	5
20. ....		95	50		80	15		70	25
24. ....		95	95		95	55		95	60
28. ....		100	95		100	80		100	75

SECOND EXPERIMENT, BEGUN APRIL 23 AND CONCLUDED MAY 22

10. ....	17	0	0	27	0	0	31	0	0
12. ....		40	20		0	0		3	0
15. ....		70	30		4	0		52	0
17. ....		70	40		15	0		65	0
20. ....		80	60		63	7		81	29
24. ....		100	60		63	15		87	42
28. ....		100	90		78	48		94	61

TABLE XIII.—*Percentage of cabbage plants developing yellows in soil with different moisture contents—average results of the two experiments shown in Table XII*

Number of days after planting.	Percentage of plants yellow.			Percentage of plants dead.		
	19 per cent moisture.	23 per cent moisture.	26 per cent moisture.	19 per cent moisture.	23 per cent moisture.	26 per cent moisture.
10. ....	0	0	0	0	0	0
12. ....	30	0	3	10	0	0
15. ....	60	5	36	15	0	0
17. ....	78	30	50	35	0	3
20. ....	88	72	75	55	11	27
24. ....	98	79	91	78	35	51
28. ....	100	89	97	93	64	68

EXPERIMENTAL DATA.—From Table XIII it may readily be seen that with favorable temperature yellows develops at any percentage of soil moisture permitting growth of the cabbage seedlings. However, it

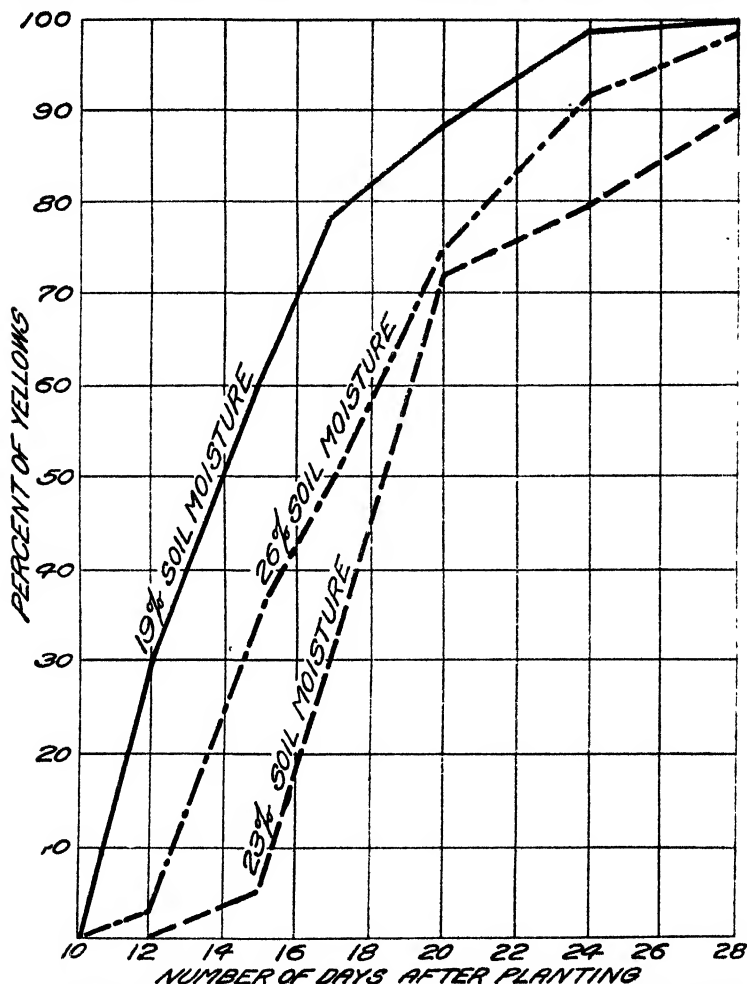


FIG. 9.—Comparison of the rate of development of yellows in Commercial Hollander seedlings given in Table XIII, grown 28 days at 23° C.

developed most rapidly and destructively in soil with 19 per cent moisture, even though, as will be shown later (Table XIV), this percentage of moisture was most favorable for the growth of the seedlings. This was a case, then, where the most vigorous plants were the least resistant to the disease.<sup>5</sup> The development of the disease with 19 per cent moisture

<sup>5</sup> Plants were grown long enough with about 15 per cent soil moisture to show that the yellows developed even more rapidly with this amount than with 19 per cent moisture. This percentage of moisture was about as low as would permit growth of the seedlings.

was very rapid until most of the plants were affected, after which the remaining plants showed infection more slowly. Conversely, the disease developed slowly but at a fairly uniform rate with 23 and 26 per cent moisture, and at the conclusion of the experiment the percentage of

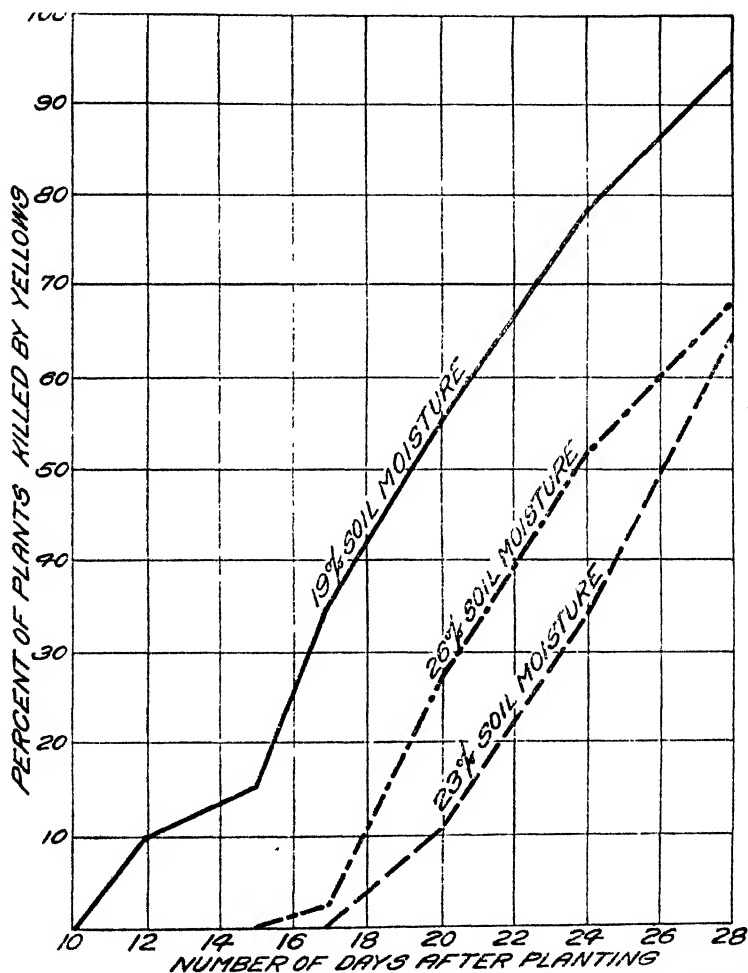


FIG. 10.—Comparison of death rate from yellows in Commercial Hollander seedlings given in Table XIII grown 28 days at 23° C.

disease was noticeably lower, especially with 23 per cent moisture. It was rather surprising to find that this comparatively slight difference in percentage of moisture made such a significant difference in the rate of development of yellows.

The difference in death rate of the affected plants is even more striking. At the conclusion of the experiment the number of plants killed by yel-

lows at 19 per cent moisture was 29 per cent greater than at 23 per cent moisture and 25 per cent greater than with 26 per cent moisture. At first sight, this condition suggests that because of the reduced functional root system the affected plants were unable to take up the necessary amount of water from the soil with 19 per cent moisture. These results are probably partially explainable on this basis, but the more rapid death rate with 26 per cent moisture than with 23 per cent and the general appearance of the plants in the former case suggest the operation of some factor in addition to deficient water supply. The fact that beyond a certain point both the general vigor of the highly susceptible plants and the percentage of yellows in them decreased with an increase in percentage of soil moisture suggests a reduction in the virulence of the *Fusarium*. It is possible, therefore, that the reduced oxygen supply in the soil due to increased water content affects both host and parasite.

#### INFLUENCE OF SOIL MOISTURE UPON THE GROWTH OF CABBAGE SEEDLINGS

It is interesting to note in this connection the influence of soil moisture upon the growth of cabbage seedlings themselves. This investigation was made simultaneously with the study of the influence of soil moisture upon the occurrence of yellows. The dry weight of the plants was used as an index of growth in this case, as previously, and the plants grown in sterilized soil as controls for the second test in the study of the disease were used for the determinations. The plants were cut at the surface of the soil 35 days after seeding and dried for 16 hours at 95° C. Six plants from each of the two receptacles were used. The average weight per plant of the 12 plants is given in Table XIV.

TABLE XIV.—Dry weight of cabbage seedlings grown in soil with different moisture contents

Soil moisture (per cent) . . . . .	19	23	26
Dry weight per plant (gm.) . . . . .	0.478	0.343	0.251

It will be seen from the foregoing table that the soil with 19 per cent moisture was the most favorable for increase in dry weight. Also, the vigor of the plants, rich leaf color, and extensive root system indicated that the plants were in a healthy, flourishing condition. Contrasted with these, the plants growing in soil with 26 per cent moisture showed decided stunting, yellowish-green leaf color, and a greatly reduced root system. In addition, the roots were brown and small. Both the discoloration and the disintegration were even more pronounced in roots grown in the "sick" soil, probably because of the action of secondary organisms following the *Fusarium*. The plants grown in the soil with 23 per cent moisture exhibited characters between these extremes.

The results of these experiments show that cabbage is not a high-moisture-loving plant and that the *Fusarium* is more pathogenic in soil with low moisture content.

#### SUMMARY

(1) Cabbage yellows, a disease caused by the vascular parasite *Fusarium conglutinans*, has been observed to develop in its most destructive form in southeastern Wisconsin only under conditions of hot, dry weather,

whereas there is little or no development even in the "sickest" soils during moist, cool weather.

(2) Hot, dry weather also retards the growth of cabbage plants.

(3) During such critical periods they become pale green in color and show a general lack of vigor; even the resistant Wisconsin Hollander shows a considerable percentage of incipient yellows. With the return of favorable rainfall and lower temperature the resistant plants overcome the attack of yellows and produce marketable heads.

(4) Such field observations soon convince one that the occurrence and severity of yellows are closely correlated with the influence of soil temperature and soil moisture, and the question arises as to how far and in what way these influences relate to the host on the one hand and to the parasite on the other.

(5) The commercial varieties have shown considerable differences in degree of resistance to *Fusarium*. Also, within any one variety there is always a variation in resistance as between individuals. During severe seasons most of the plants of the standard commercial varieties quickly succumb to the disease; others linger along in a dwarfed condition, slowly shedding their lower leaves, whereas a few scattered individual plants in the field usually remain healthy and produce marketable heads. This variation in individual susceptibility or disease resistance has been the basis for developing the resistant Wisconsin strains.

(6) The preceding observations and experiments were made upon plants which had been transplanted into the field.

(7) Gilman (8), in his greenhouse experiments, showed that yellows did not develop below  $17^{\circ}\text{C}$ . while it developed quite destructively at  $23^{\circ}$  to  $25^{\circ}$ . He also studied the effect of certain temperatures upon the growth of the fungus in culture, but he did not define the upper temperature limits either for its growth or for the occurrence of the disease.

(8) The writer undertook to learn more exactly the factors regarding these questions, using seedling cabbage plants. The purposes outlined were: (1) To determine the range of soil temperature for the occurrence of yellows in cabbage seedlings, the air temperature being kept constant; (2) to study the influence of soil temperature upon the growth of cabbage seedlings in noninfested soil; (3) to determine in like manner the influence of soil moisture both upon the growth of cabbage seedlings and upon the occurrence of yellows in them; (4) to study the influence of high soil temperature and soil moisture upon the susceptibility of the resistant strain, that is, upon the "breaking down" of resistance. These experimental investigations have justified several conclusions bearing upon these questions as follows.

(9) Pure cultures of *Fusarium conglutinans* on potato agar plates showed the following relations to temperature: (1) The organism grew at temperatures ranging from  $7^{\circ}$  to  $35^{\circ}\text{C}$ .; (2) the optimum temperature, using diameter of colony as a criterion, at the end of 7 days was  $25^{\circ}$  to  $27^{\circ}$ ; (3) although no growth took place in 7 days at  $37^{\circ}$ , the organism was not killed at this temperature.

(10) Cabbage yellows develops in seedlings growing in "sick" soil at soil temperatures ranging from  $17^{\circ}$  to  $35^{\circ}\text{C}$ . At  $17^{\circ}$  it develops very slowly even in the most susceptible strains. In naturally infested soil the disease appears first and develops most rapidly in both resistant and susceptible strains at  $26^{\circ}$  to  $29^{\circ}$  and in sterilized artificially inoculated soil at  $29^{\circ}$  to  $32^{\circ}$ .



(11) It thus appears that the optimum temperature for the vegetative growth of the fungus in culture practically coincides with the optimum for the development of yellows in seedlings. This is above the optimum for the growth of the host plant.

(12) Cabbage seedlings grew at all temperatures from 14° to 38° C., but only very poorly at the latter. At 38° the seedlings emerged from the soil, but most of them died before developing any true leaves. The optimum soil temperature for seedling growth was found to be about 20° when the Wisconsin tank method was used.

(13) Soil temperature greatly influences the length of the incubation period for the disease. Under controlled conditions the incubation period varied from 18 days at 17° C. to 8 days at 29° to 32°.

(14) With constant favorable temperature yellows developed at any percentage of soil moisture permitting growth of cabbage seedlings. In soil with a moisture-holding capacity of 46 per cent the yellows developed more rapidly and destructively in susceptible plants when the moisture was held at 15 per cent than at 19, 23, or 26 per cent. At 19 per cent moisture the disease appeared two days later than at 15 per cent, but once it had started the subsequent rate of development was about the same in both cases.

(15) Nineteen per cent soil moisture was the most favorable for the growth of cabbage seedlings when the soil temperature was held at 23° C. and the air at 14° to 18°. The growth of plants was materially checked at 26 per cent and also at 15 per cent soil moisture. At these less favorable moistures the color of the foliage was quite abnormal. Plants grown at 19 per cent soil moisture had healthier color and a more extensive root system, and the dry weight was almost twice that at 26 per cent moisture. Thus it is evident that the soil moisture (15 per cent) which was too low for good growth of the host plant was most favorable for the development of yellows, while the soil moisture (19 per cent) which proved almost equally as stimulating to the disease was highly favorable for normal development of the host plant.

(16) The preceding conclusions relative to the relation of soil temperature and soil moisture to the occurrence of yellows in cabbage seedlings were first worked out in the greenhouse under experimentally controlled conditions.

(17) Soil temperature and soil moisture influenced the occurrence of yellows in the field in a manner similar to that in the greenhouse. With seedlings started in May, June, and September when the soil temperature was low, the disease showed a lower percentage and was slower in appearing than with those started in July and August when a high soil temperature prevailed.

(18) These facts, no doubt, are of significance in the geographical distribution of the disease. In the Southern States, where cabbage is generally grown commercially as a winter or early spring crop, the soil temperature is very probably too low for the organism to gain a foothold on such plants. Reports from these sections indicate, however, that where once introduced the *Fusarium* establishes itself on summer-grown cabbage or other related hosts and may be expected to persist and attack the crops whenever soil temperature is favorable.

(19) The foregoing conclusions are based on the general or average behavior of the cabbage seedlings. It is in this connection that noteworthy variations in the incubation period as between certain individual plants in the same receptacle may be as great as between some plants

grown at 17° and at 29° C. This variation occurs not only with seedlings of the susceptible strain but also with those of the Wisconsin Hollander.

(20) The great difference in the individuality of plants, as shown by the variation in the length of incubation period of the disease and degree of infection of plants, is conclusive evidence of a variation in degree of resistance. This variation in degree of resistance is, no doubt, due to a lack of factors or to a heterozygous condition of some of the plants for the factors for resistance.

(21) The degree of resistance shown by a strain of cabbage depends to a considerable extent upon the environmental conditions under which the plants are grown. In our trials all plants remained healthy in "sick" soil below 17° C.; many were resistant at 17° to 23°, and a small number were resistant at the higher temperatures. At all temperatures within the infection range some plants showed intermediate degrees of resistance, whereas others were entirely susceptible. Somewhat similar results were obtained with different percentages of soil moisture.

(22) *Fusarium* resistance in cabbage becomes more pronounced with increasing age of the plant. Young seedlings of the resistant strain, Wisconsin Hollander, developed a relatively high percentage of yellows when started in "sick" soil at high temperatures, whereas plants grown for 30 days or more in noninfested soil, or even in "sick" soil below 17° C., when transferred to "sick" soil at high temperatures developed only a low percentage of yellows and then usually only in an incipient form.

(23) This fact has practical significance in predicting, interpreting, or improving the performance of this and the other resistant Wisconsin strains in the following ways:

(a) In the first place, it indicates that the strains will give the best results commercially when started in a noninfested seed bed during cool spring weather. This accords with the best practice of commercial cabbage growers in the Northern States.

(b) In the second place, it indicates that these resistant strains may safely be recommended for trial in *Fusarium* "sick" soil in all geographic localities where the prevailing temperatures at the different early stages of development of the cabbage plants are not distinctly higher than those in Wisconsin.

(c) Finally, the resistance of these Wisconsin strains may be expected to "break down" in some degree proportional with the elevation of temperature above this point. However, in the Northern States, even in the warmer seasons, this usually stops with the incipient stages of the disease and leaves the crop commercially successful. If more trying conditions are met with elsewhere, it seems probable that through further selection strains showing a correspondingly higher degree of resistance may be secured.

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**PLATE 1**

**A.—Comparison of shoots of Wisconsin Hollander cabbage seedlings grown during November and December for 53 days from seed in noninfested soil at the temperatures indicated.**

**B.—Comparison of root systems of plants shown in A.**

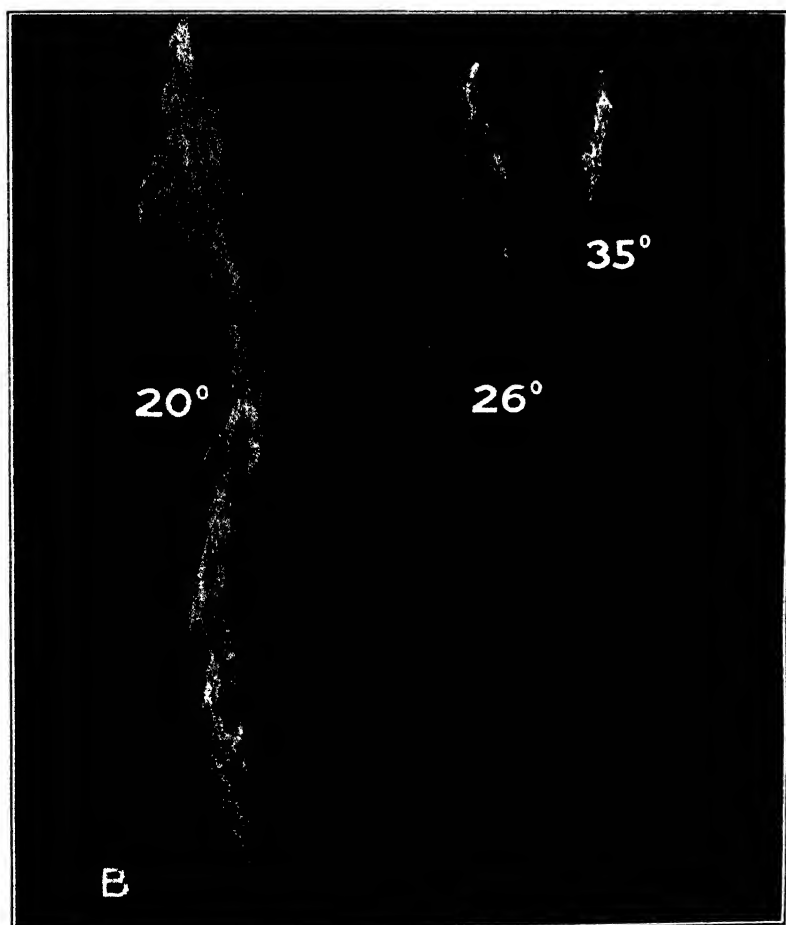
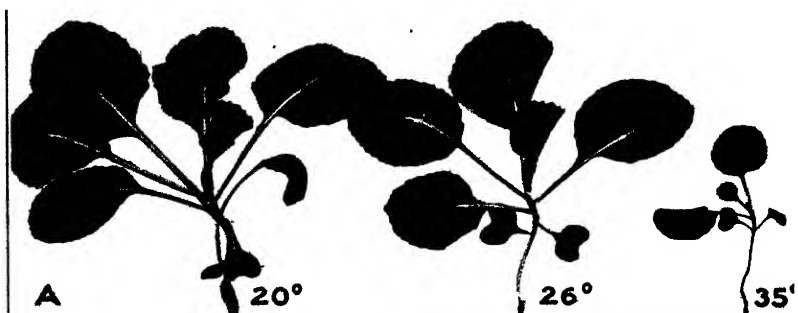




PLATE 2

A.—Commercial Hollander plants grown 30 days from seed in sterilized soil at 17° C. All 10 plants healthy.

B.—Commercial Hollander plants grown 30 days from seed in "sick" soil at 17° C. Three of the 10 dead; all others yellowing and stunted.

C.—Wisconsin Hollander plants grown 30 days from seed in "sick" soil at 17° C. All healthy.

D.—Susceptible Commercial Hollander plants grown 36 days from seed in non-infested soil, then 30 days after transplanting to "sick" soil at 15° C. All healthy.

E.—Plants of same variety as those in D and grown under same conditions but transplanted to "sick" soil at 17° C. Two dead, the other yellow and stunted.





# ACTION OF SOAP UPON LEAD ARSENATES<sup>1</sup>

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Soap is sometimes added to the water used for applying lead arsenate as an insecticide. Few authorities advise its use on apple trees or tender vegetation, but it is occasionally advised for use upon hardy and smooth-leaved crops, such as cabbage and sugar beets. It is said that the use of soap with arsenates is increasing.

## THE ADVANTAGES

Several advantages are to be gained by the use of soap. The lead arsenate remains in suspension longer in soap solution than in pure water, hence is more easily and evenly applied, especially when hand-operated sprayers are used. The soap also helps to spread the arsenical. J. R. Parker (3)<sup>2</sup> found that soap retarded the settling of lead arsenate and stated that it also improved the spreading upon smooth-leaved plants.

## THE DISADVANTAGES

The disadvantage to be feared is that of burning the leaves of the crop sprayed by arsenic dissolved by the action of the soap upon the lead arsenate.

Tartar and Bundy (6) in 1913 reported that fruit trees were injured by spraying with soap and lead arsenate and showed that the use of the soap increased the quantity of soluble arsenic in the liquid. They also noted that acid arsenate was much more soluble in soap solution than neutral arsenate. Headden (1) had already pointed out the danger in using water containing alkali salts for arsenical sprays. It was therefore natural that some presumed that the solubility of lead arsenate in soap solution was due to free sodium carbonate in the soap, and that the damage could be avoided by using only neutral soaps. However, it has been commonly known to chemists for a long time that lead readily forms insoluble soaps. Therefore it is to be expected that neutral soaps might undergo double decomposition with the lead arsenate, forming lead soap and alkali arsenates which would be soluble. This possible reaction may be represented thus:

Sodium soap + lead arsenate = sodium arsenate + lead soap. As the sodium compounds are soluble and the lead compounds are insoluble, it is not to be expected that the reaction will go to completion in either direction. This double decomposition, however, is not the only imaginable reaction which might take place between the soap and lead arsenate; and, in fact, others have been reported by some investigators and will be discussed later. So far as observed by the writer, no one has heretofore reported whether or not soaps of different fatty acids behaved differently toward lead arsenates.

<sup>1</sup> Accepted for publication May 29, 1922.

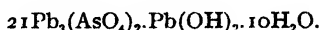
<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 95.

## FORMULAS OF LEAD ARSENATES

The composition and naming of lead arsenates is a matter that was at one time in great confusion. It has been held by most chemical workers in this field, however, that the lead arsenates on the market fall into three classes:

1. Triplumbic ortho arsenate ( $\text{Pb}_3(\text{AsO}_4)_2$ ), called by manufacturers "ortho," "triplumbic," "normal," or "neutral" lead arsenate.
2. Diplumbic ortho arsenate ( $\text{PbHAsO}_4$ ), called "monoplumbic," "diplumbic," "acid arsenate," or simply "lead arsenate."
3. Mixtures of these two compounds.

Tartar and Robinson (4, 7) believe that the substances here designated as class 1 are really of much more complicated structure and suggest the formula

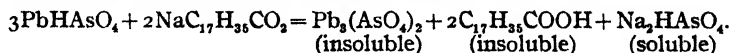


They also state that the diplumbic arsenate,  $\text{PbHAsO}_4$ , can be readily transformed into this basic compound by treating with ammonia and that by this treatment, a definite quantity of arsenic is dissolved and may be recovered in the ammonia solution. It thus is an accurate method for the determination of the quantity of diplumbic arsenate in class 3.

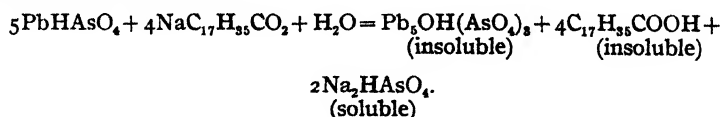
Their formula for this basic compound was refuted in two papers appearing at the same time by McDonnell and Smith (2) and by G. Ennis Smith (5), both suggesting the same formula,  $\text{Pb}_3\text{OH}(\text{AsO}_4)_2$ , and G. Ennis Smith suggesting the name lead hydroxy arsenate. By this treatment with ammonia, 40 per cent of the arsenic present in the diplumbic goes into solution and the rest remains insoluble as the basic compound.

All investigators agree that the diplumbic arsenate is the more active compound of the two and that where conditions are such as to cause any apprehension of damage from burning the normal compound should be preferred. On the other hand, the acid arsenate contains a greater percentage of arsenic; hence the consumer can secure the required quantity of arsenic with a smaller number of pounds to pay freight and profits upon. The acid arsenate is sold in much greater quantity in many markets.

G. Ennis Smith, in the paper already referred to (5), states that soap acts upon diplumbic arsenate in the way that Tartar and Robinson reported for ammonia (4, 7)—transforms it into the more basic lead arsenate and dissolves some of its arsenic but sets free an equivalent quantity of the fatty acid, which is insoluble. Thus assuming that the end product is triplumbic arsenate, the reaction is:



In this case 33 1/3 per cent of the arsenic of the diplumbic arsenate is made soluble. In case we consider the end product to be lead hydroxy arsenate, the reaction is:



In this case 40 per cent of the arsenic of the diplumbic arsenate goes into solution. Under this theory there should be no reaction possible

between soap and the lead hydroxy arsenate; hence treating that compound with soap should not bring arsenic into solution.

McDonnell and Smith (2) state that triplumbic arsenate is formed under certain conditions, but that it has a very limited range of existence and is readily changed over to the more basic lead arsenate (lead hydroxy arsenate) by treatment with ammonia. By that change 10 per cent of its arsenic should go into solution; hence that should be the maximum possible to obtain by treatment with soap if the end product is in fact lead hydroxy arsenate.

On the other hand, if the reaction between soap and lead arsenate is of the simple double decomposition type, it might be possible for diplumbic arsenate to yield more than the  $33\frac{1}{3}$  or 40 per cent above quoted, and it might be that the lead hydroxy arsenate would yield soluble arsenic. Furthermore, whichever the type of the reaction, its completeness might be different with soaps of different fatty acids. With these points in view, the writer's materials were selected as follows:

#### THE SOAPS

The soaps were prepared in the laboratory from c. p. sodium hydroxid and the best grade of the free fatty acids, stearic and oleic. Palmitic was omitted, as no satisfactory palmitic acid was at hand. After the soaps had been prepared they were analyzed for free alkali and for moisture. The stearic soap contained 83.1 per cent moisture and 0.85 per cent free alkali, computed as sodium carbonate. It was weighed in this form for the first series of trials but is recorded on the dry basis. For the second series this soap was dried to 4.3 per cent moisture and weighed in that form.

The oleic soap contained 83.5 per cent moisture and no free alkali. Though it was not found practicable to dry the oleic soap, on the tables it is reported on the dry basis.

#### THE LEAD ARSENATES

The lead arsenates were selected from a large number of samples that had been furnished by manufacturers for comparison and are designated in this paper as "M" and "T". "M" was labeled by the manufacturer "Monoplumbic Lead Arsenate" and contained at the time of the first series of tests 41.9 per cent water. When dry, it contained arsenic equivalent to 31.53 per cent  $As_2O_5$ , and lead to 65.4 per cent PbO. When received, it was analyzed for water soluble arsenic and found to contain, on the dry basis, 0.85 per cent  $As_2O_5$ . This was taken to indicate that this arsenical was a practically pure diplumbic hydrogen arsenate. A later test on the dried material with 4 per cent ammonium hydroxid, in the manner described by Tartar and Robinson (4, 7), dissolved 8.85 per cent  $As_2O_5$ , indicating that only about 67 per cent of the material was really diplumbic arsenate ( $PbHAsO_4$ ) and that 30 per cent of the arsenic present was in some other form.

"M" was used in paste form for the first series and in dry form for the second.

"T" was labeled "Triplumbic Ortho Lead Arsenate" and contained 46 per cent water, and in the dry material arsenic to the amount of 25.75 per cent  $As_2O_5$ , and lead to 72.10 per cent PbO. The water-soluble arsenic when received was equivalent to 0.43 per cent  $As_2O_5$ , on the dry basis. This was supposed to indicate that the paste was as

labeled, a pure triplumbic arsenate; but since the authorities already noted cast considerable doubt upon the existence of this salt in commercial lead arsenates, it might have been a mixture. The dry matter when treated with 4 per cent ammonium hydroxid, yielded but 0.22 per cent of soluble  $\text{As}_2\text{O}_5$ , indicating but 1.70 per cent  $\text{PbHAsO}_4$ .

#### BRINGING THE MATERIALS IN CONTACT

Preliminary work indicates that when the soap solution is mixed with the lead arsenate, either as paste or dry, it is difficult to get concordant results for the quantity of arsenic made soluble. It was also noted that when finely divided lead arsenates were shaken with soap solutions the resulting precipitates were bulky and the individual curds frequently large. Besides, as would be expected of either lead soaps or free fatty acids, they were water-repellent. It seemed probable, therefore, that the particles of lead arsenate, when brought into contact with the soap solution, were at once acted upon at the surface and surrounded with an envelope of the water-repellant reaction product, which in turn protected the lead arsenate at the interior of the curd from further action. This reasoning led to the following procedure.

In each case, the treatment was carried out with 2 liters of soap solution in a 2½-liter acid bottle. The soap to be used for each sample was dissolved in 1 liter of distilled water, to be later mixed with a second liter of distilled water, as follows: The water was placed in the acid bottle and the weighed sample of lead arsenate was placed in a porcelain mortar where it was ground with successive portions of the soap solution. The soap and finely divided material was poured into the liter of water, taking care that the coarser particles were left in the mortar to be ground with the next portion of the soap solution. When all the lead arsenate had become so fine as to pass easily into the water with the soap solution, the remainder of the liter of soap solution was poured into the bottle, making 2 liters. The bottle was stoppered and shaken at intervals for five days, then filtered through paper, using suction. One half the samples were treated as stated; the other half were ground in the water and poured into the soap solution, the only difference being the fluid used for the grinding.

The lead arsenates in the first series were weighed in paste form, 2 gm. of "M" amounting to 0.346 gm. of  $\text{As}_2\text{O}_5$  and to 0.707 gm.  $\text{PbO}$ , and 2.15 gm. of "T" amounting to 0.289 gm. of  $\text{As}_2\text{O}_5$  and to 0.805 gm.  $\text{PbO}$ .

The soaps were used in two concentrations, 0.6 gm. and 1.8 gm. (dry basis) per 2 liters of solution.

The filtrate thus secured was analyzed for arsenic, while the insoluble material was dried, removed from the filter, and preserved for analysis for lead and for total arsenic.

It was found advisable to remove the soap that remained dissolved in the filtrate. This was accomplished as follows: The measured aliquot to be analyzed was placed in a beaker and a few drops of barium chlorid solution were added, just enough being used to clear the solution. The insoluble barium soap separated at once and was removed by filtering, leaving a clear solution that could be reduced and titrated with iodine in the usual Gooch and Browning method (*8*, p. 239).

The quantity of arsenic rendered soluble and found in the filtrate is given in Table I.

TABLE I.—Percentage of arsenic made soluble by treatment of lead arsenate paste with soap—first series

No.	Soap.		Lead arsenate.		As <sub>2</sub> O <sub>3</sub> in 200 cc	As <sub>2</sub> O <sub>3</sub> rendered soluble.	Solution in which lead arsenate was ground.
	Quantity. (dry).	Kind.	Quantity <sup>1</sup>	Brand.			
	Gm.		Gm.		Gm.	Per cent.	
1	0.6	Sodium stearate	2.0	"M"	0.01760	50.8	Water.
2	1.8	do	2.0	"M"	0.01870	54.0	Do.
3	.6	do	2.0	"M"	0.01424	47.1	Soap.
4	1.8	do	2.0	"M"	0.01704	49.2	Do.
5	.6	do	2.15	"T"	0.00884	32.7	Water.
6	1.8	do	2.15	"T"	0.00573	19.8	Do.
7	.6	do	2.15	"T"	0.00670	23.2	Soap.
8	1.8	do	2.15	"T"	0.00752	26.0	Do.
9	.6	Sodium oleate	2.0	"M"	0.00335	9.7	Water.
10	1.8	do	2.0	"M"	0.00817	23.6	Do.
11	.6	do	2.0	"M"	0.00251	7.3	Soap.
12	1.8	do	2.0	"M"	0.00726	21.0	Do.
13	.6	do	2.15	"T"	0.00111	3.8	Water.
14	1.8	do	2.15	"T"	0.00111	3.8	Do.
15	.6	do	2.15	"T"	0.00084	2.8	Soap.
16	1.8	do	2.15	"T"	0.00084	2.8	Do.

<sup>1</sup> Pastes contained:

	"M"	"T"
As <sub>2</sub> O <sub>3</sub> . . . . .	17.30 per cent.	13.40 per cent.
PbO . . . . .	35.35 per cent.	37.45 per cent.

TABLE II.—Percentage of arsenic made soluble by treatment of dry lead arsenate with soap—second series

No.	Soap		Lead arsenate <sup>1</sup>		As <sub>2</sub> O <sub>3</sub> in 200 cc.	As <sub>2</sub> O <sub>3</sub> rendered soluble.	Solution in which lead arsenate was ground.
	Quantity (dry).	Kind.	Quantity.	Brand.			
	Gm.		Gm.		Gm.	Per cent.	
17	0.6	Sodium stearate	1.35	"M"	0.01280	30.1	Water.
18	1.8	do	1.35	"M"	0.02814	66.2	Do.
19	.6	do	1.35	"M"	0.01325	31.1	Soap.
20	1.8	do	1.35	"M"	0.03097	72.9	Do.
21	.6	do	1.20	"T"	0.00625	20.2	Water.
22	1.8	do	1.20	"T"	0.01084	35.1	Do.
23	.6	do	1.20	"T"	0.00596	19.3	Soap.
24	1.8	do	1.20	"T"	0.00893	28.9	Do.
25	.6	Sodium oleate	1.35	"M"	0.00144	3.4	Water.
26	1.8	do	1.35	"M"	0.00345	8.1	Do.
27	.6	do	1.35	"M"	0.00144	3.4	Soap.
28	1.8	do	1.35	"M"	0.00308	7.2	Do.
29	.6	do	1.20	"T"	0.00122	3.9	Water.
30	1.8	do	1.20	"T"	0.00066	2.1	Do.
31	.6	do	1.20	"T"	0.00122	3.9	Soap.
32	1.8	do	1.20	"T"	0.00081	2.6	Do.

<sup>1</sup> Dry arsenate contained:

	"M"	"T"
Moisture . . . . .	0	0.24 per cent.
As <sub>2</sub> O <sub>3</sub> . . . . .	31.50 per cent.	25.75 per cent.
Lead (PbO) . . . . .	65.40 per cent.	72.10 per cent.
As <sub>2</sub> O <sub>3</sub> soluble in 4 per cent ammonia . . . . .	8.85 per cent.	0.22 per cent.

The second series was in most respects a repetition of the first, except that the lead arsenates were dry and used in slightly larger quantities per sample. The sodium stearate soap was also almost dry in this case, but the quantity of soaps used (dry basis) was the same as before.

The samples, treatment, and soluble arsenic found are given in Table II.

The grinding in the water or in the soap solution was perhaps more thorough than in the first series, but the shaking was continued for only four days.

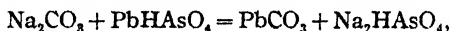
It will be noted that the pair of tests 5 and 6, in Table I, are not in agreement with other pairs of similar tests. The general tendency is, the greater the concentration of soap, the greater the quantity of soluble arsenic found, but in this one pair this tendency seems to be reversed. It is believed this indicates some mistake, possibly in the numbering of the bottles, or of the record. The corresponding pair in Table II, No. 21 and 22, is in accord with the tendency noted.

#### EFFECT OF THE LIQUID USED FOR GRINDING

Comparison of the quantity of arsenic made soluble in pairs of tests otherwise identical, but in one of which the grinding was in the presence of pure water and the other in the presence of soap solution, shows that the quantity of arsenic made soluble is seldom the same. It frequently varies by several per cent of the arsenic present, but does not always vary in the same direction. The cause of this lack of uniformity was not determined, but conceivably might be as follows: The shells of lead soap formed about the small solid particles of lead arsenate are somewhat plastic and the real effect of the grinding might be a "churning" one that would serve to stick some of the particles together and increase the protective action of the shells of lead soap. In other cases the grinding might remove the coating of lead soap, as was the intent when the work was done.

#### SOLUBLE ARSENIC DUE TO ACTION OF FREE ALKALI DEDUCTED

Since the free alkali in the sodium stearate soap would probably exert some solvent action, it is worth while to compute how much of the soluble arsenic found might be due to that cause. Starting with the reaction



the highest ratio possible between sodium carbonate and soluble arsenic pentoxid is 1 to 1.085. On this basis Table III is computed from the data already given, showing the quantity of sodium carbonate present, the equivalent quantity of  $\text{As}_2\text{O}_5$  that might be made soluble, the quantity of soluble arsenic as actually found, and, by difference, the quantity that must be made soluble by the neutral soap, the quantity of  $\text{As}_2\text{O}_5$  present in the lead arsenate used, and the percentage made soluble by the action of the soap aside from the free alkali.

Since the oleic soap contained no free alkali, no similar table is presented for correction of the results on oleic soap in Tables I and II.

TABLE III.—Quantity of  $As_2O_3$  that might be made soluble by the free (excess) sodium carbonate with stearate soap (4.72 per cent)

No.	Brand of lead arsenate.	Weight of free $Na_2CO_3$	Equivalent weight of $As_2O_3$	$As_2O_3$ found in 2 liters.	Quantity due to soap (by difference).	Total $As_2O_3$ in system.	Arsenic made soluble by neutral soap.
		Gm.	Gm.	Gm.	Gm.	Gm.	Per cent.
1.....	"M"	0.0095	0.0103	0.1760	0.1657	0.3460	47.89
2.....	"M"	.0285	.0309	.1870	.1561	.3460	45.12
3.....	"M"	.0095	.0103	.1424	.1321	.3460	38.18
4.....	"M"	.0285	.0309	.1704	.1397	.3460	40.37
5.....	"T"	.0095	.0103	.0884	.0781	.2890	27.02
6.....	"T"	.0285	.0309	.0573	.0264	.2890	9.13
7.....	"T"	.0095	.0103	.0670	.0567	.2890	19.62
8.....	"T"	.0285	.0309	.0572	.0261	.2890	9.02
17.....	"M"	.0095	.0103	.1280	.1177	.4050	29.06
18.....	"M"	.0285	.0309	.2814	.2505	.4050	61.85
19.....	"M"	.0095	.0103	.1325	.1222	.4050	30.17
20.....	"M"	.0285	.0309	.3097	.2788	.4050	68.84
21.....	"T"	.0095	.0103	.0625	.0522	.3090	16.89
22.....	"T"	.0285	.0309	.1084	.0775	.3090	25.08
23.....	"T"	.0095	.0103	.0596	.0493	.3090	15.95
24.....	"T"	.0285	.0309	.0895	.0586	.3090	18.96

It is plain from Tables I, II, and III that "M" is very much more acted upon by both soaps than is "T" and that sodium stearate is very much more (two to seven times) effective in dissolving arsenic from lead arsenates than is sodium oleate. It follows that if it is desired to use soap with lead arsenate in spraying, the danger of injury from arsenic in solution can be diminished in some degree by securing triplumbic arsenate but could be almost entirely overcome by using only sodium oleate as the soap.

It is also clear that sodium stearate at least does not act as stated by G. Ennis Smith (5), but dissolves arsenic from the basic arsenate and dissolves much more than the 40 per cent that should be made soluble in transforming diplumbic into lead hydroxy arsenate. This behavior is exactly in accord, however, with the supposition that the reaction is of the double decomposition sort, giving insoluble lead soaps as one of the end products, instead of free fatty acids as Smith states. In order to confirm this supposition, the insoluble residues from the first series were analyzed for arsenic and for lead.

In case the reaction stops as soon as the lead hydroxy arsenate is reached, the ratio of lead oxid to arsenic pentoxid could never be greater than that in the lead hydroxy arsenate, 1 to 3.23, and would usually be lower than that, as there would be some material unacted upon. On the other hand, if the reaction is of the double decomposition sort, it might be possible to carry the reaction far beyond that ratio, as in fact occurs in six of the eight samples that were acted upon by sodium stearate. When oleate soap was used, the reaction was so incomplete that the ratio never approaches even 1 to 3. It may be that soap first transforms diplumbic arsenate into lead hydroxy arsenate and then undergoes a double decomposition with that salt, but it is hard to explain the results on the supposition that the action stops at the lead hydroxy arsenate. The results of the analysis of the dry insoluble residue and the ratio of  $As_2O_3$  to PbO are given in Table IV.



TABLE IV.—Analysis of 0.1 gm. dry insoluble residue from treatment of lead arsenate with soap solution

No.	Soap.		Lead arsenate.		Weight of $As_2O_3$	Weight of PbO	Ratio of $As_2O_3$ to PbO
	Quantity.	Kind.	Quantity.	Brand			
	Gm.		Gm		Gm.	Gm.	
1	0.6	Sodium stearate....	2.00	"M"	0.0101	0.0437	1 : 4.3
2	1.8	..do .....	2.00	"M"	.0065	.0274	1 : 4.2
3	.6	..do .....	2.00	"M"	.0122	.0460	1 : 3.8
4	1.8	..do .....	2.00	"M"	.0065	.0283	1 : 4.3
5	.6	..do .....	2.15	"T"	.0132	.0475	1 : 3.6
6	1.8	..do .....	2.15	"T"	.0092	.0310	1 : 3.4
7	.6	..do .....	2.15	"T"	.0140	.0468	1 : 3.3
8	1.8	..do .....	2.15	"T"	.0086	.0257	1 : 3.0
9	.6	Sodium oleate ...	2.00	"M"	.0256	.0538	1 : 2.1
10	1.8	..do .....	2.00	"M"	.0211	.0482	1 : 2.3
11	.6	..do .....	2.00	"M"	.0322	.0552	1 : 1.7
12	1.8	..do .....	2.00	"M"	.0204	.0470	1 : 2.3
13	.6	..do .....	2.15	"T"	.0244	.0635	1 : 2.6
14	1.8	..do .....	2.15	"T"	.0242	.0610	1 : 2.5
15	.6	..do .....	2.15	"T"	.0221	.0564	1 : 2.5
16	1.8	..do .....	2.15	"T"	.0185	.0482	1 : 2.6

Theoretical for diplumbic ortho arsenate ( $PbHAsO_4$ ) 1 1.04Theoretical for triplumbic ortho arsenate ( $Pb_3(AsO_4)_2$ ) 1 2.91Theoretical for lead hydroxy arsenate ( $Pb_3OH_4(AsO_4)_3$ ) 1 3.33

## SUMMARY

The data exhibited make the following conclusions plain

(1) That pure soaps dissolve arsenic from both samples of lead arsenate and therefore might cause injury to foliage.

(2) That sodium stearate is much more effective in dissolving arsenic from both "M" and "T" than is sodium oleate, from two to six or even seven times as much soluble arsenic being found in the solution of the former as in the latter.

(3) That, as Tartar and Robinson point out (4, 7), the arsenic of diplumbic arsenate is much more acted upon than the more basic compound.

(4a) Increasing the concentration of the stearic soap solution increased the amount of arsenic made soluble.

(4b) Increasing the concentration of the oleic soap made more arsenic soluble from the acid lead arsenate "M" but did not increase the amount made soluble from the basic lead arsenate "T."

(5) The extent of the action was sometimes greater when the lead arsenate was ground in water than in the soap, sometimes less. No definite statement can be made as to which is most effective.

(6) Sodium stearate dissolves far too much arsenic from diplumbic arsenate to confirm the supposition that its action stops with the conversion of diplumbic into lead hydroxy arsenate.

(7) Both sodium stearate and sodium oleate dissolve arsenic from basic lead arsenate, the stearic soap in large quantities (as much as 25 per cent).

(8) Numbers 6 and 7 together indicate that the action of soaps upon lead arsenates is of the double decomposition sort.

(9) Danger of injury from soluble arsenic in spraying with lead arsenates and soap can be largely eliminated if the soap is entirely made from oleic acid.

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## PHYSIOLOGICAL REQUIREMENTS OF ROCKY MOUNTAIN TREES<sup>1</sup>

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### I. INTRODUCTION

The most casual observer ascending any mountain range in the western United States can hardly fail to be impressed with the fact that there is a sharp line of demarcation between the grassy plains at the base of the mountain and the wooded slopes of the mountain proper. As one ascends farther, gradual or sometimes very abrupt changes are noted in the character of the forest cover. With increase in altitude the forest generally becomes more dense, trees of greater stature are observed, and those who are able to distinguish note the occurrence of new species in each altitudinal zone. To a lesser degree the same differences in the forest cover may be noted on opposing slopes at the same elevation—that is, slopes facing the south bear forests similar to those at lower elevation, while those facing the north have the character of higher altitudes. The vegetation of ridges is always different from that of ravines at the same altitudes.

The laymen will recognize that these differences in the forest cover are the result of different "growing conditions" at different elevations, just as a person who had lived in the South would recognize, almost instinctively, that the growing conditions of the mountain valleys of Colorado could not possibly be suited to the cultivation of corn or cotton.

While the intensive study of the relations of plants to the soil and climatic conditions which comprise their environment goes under the formidable name "ecology," ecological knowledge is not confined to scientists and, in fact, has been common property for ages. The writer has had opportunity to observe the first impressions of a great many people who were visiting the western mountains for the first time, and has been impressed by the amount of logic exhibited in relating cause and effect in the matter of forest distribution. Why does this southerly exposure bear an open, scrubby forest of yellow pine, and that northerly exposure, directly opposite, bear a much more dense stand of vigorously growing fir? Often the people who know no botany and much less ecology take in the situation at a glance, at least so far as it is possible to do so from superficial evidence.

To the forester such questions are of the utmost practical importance. Not only is it the forester's business to know the trees with which he

<sup>1</sup> Accepted for publication July 6, 1921.

<sup>2</sup> This project has been under the direction of the writer since its inception in 1910, and he assumes full responsibility for the quality of the work done and for the conclusions deduced from the data. It is, however, a pleasure to acknowledge the great efforts which have been required of a number of observers in the accumulation of the records. The records furnished by the Weather Bureau are duly acknowledged, and the efforts of all those numerous and changing observers who have created these records. The records of the Wagon Wheel Gap and Fremont Experiment Stations are the result of the concerted effort of many regular observers, all of whom deserve credit. The original installation of instruments at the Wagon Wheel Gap Experiment Station was made, and the records were obtained for two years, under the direction of B. C. Kadel, of the Weather Bureau.

is daily working, their relative and absolute demands for moisture, light, heat, and soil fertility, but he must be ready to make practical use of such knowledge in formulating his plans for reforestation and in deciding upon the amount and kind of timber which may safely be cut from a given area. Consciously or unconsciously he is daily making use of whatever knowledge he may possess as to the physiological requirements of each species in his region—that is, those properties of the tree which determine that it will grow best under certain conditions of soil and atmosphere. It is of the utmost importance that this knowledge should not be superficial and that through the increase in scientific facts forestry should be removed from its empiric basis.

Unfortunately, much of our knowledge of tree physiology and tree requirements is still very vague, and it is the aim of forest ecology to increase, systematize, and analyze such knowledge. As a means of systematizing the knowledge of trees, in this report we speak of an area of forest of essentially uniform composition as a forest type; and it is assumed, since the composition of the forest is uniform over a given area, that the environmental conditions which have brought this uniform forest into existence must be just about the same over the whole area, or at least must have been the same at the time when the forest started. Usually a forest type is given the name of the tree species which predominates and gives it its essential character, even though a great many other species may occur in the same stand in lesser numbers. Thus a forest in which Douglas fir is the most prominent tree, with occasional neighbors of spruce and pine, would be spoken of as a "Douglas fir type." The word "type" is somewhat loosely used to refer to the ground occupied as well as to the forest itself.

A broader use of the word "type" is as a synonym for forest zone or altitudinal zone. It is true that the character and composition of the forest changes gradually with a change in altitude, and, for example, most of the ground between elevations of 8,000 and 10,000 feet, in a given region, might be occupied by Douglas fir stands. But, from the lower extremity, a great many strips of yellow pine forest might extend into this zone on the warmer, southerly exposed areas, and likewise from the upper edge there would extend belts of spruce. As a general term, therefore, the word "zone" is far preferable to "type," and the latter will be used in this report only for specific areas bearing forests of uniform character.

As a further distinction between forest areas, the forester has brought into use the word "site" to describe the producing qualities of the ground with respect to any particular species. Thus among many Douglas fir types, all of which were characterized by the predominance of Douglas fir over other species, it might be desirable to distinguish those of the best quality as "Douglas fir sites I" and those of very meager producing qualities as "Douglas fir sites III." Such distinctions are usually based upon the evidence of the tree growth itself—that is, either the apparent rate of growth determined, for example, by the general vigor of the stand, or the growth rate actually measured, is the best possible evidence of the quality of the ground. But there is every reason to believe that the productive capacity of the ground can be measured, sooner or later, in terms of the soil quality and the atmospheric conditions which simultaneously affect tree growth, so that any systematic effort to study forest types and to describe those qualities which distinguish them must inevitably be at the same time a study of sites.

## OBJECTS OF THE PRESENT STUDY

Having now shown in a general way the nature of the problem which is constantly under consideration by students of ecology and forestry, the purpose of the study (one phase of which is covered by the present paper) may be definitely stated. As a matter of fact, it has several purposes, which are by no means distinct, but worthy of individual enumeration.

1. To compare the environments of the several species of trees with respect to each condition which may be separately measured, in order to determine what particular conditions have the most important bearing on the initiation of new stands, favoring one species rather than another.

2. After noting the differences in the conditions of the various types, which really indicate differences in the physiological functioning of the species, to determine experimentally the degree of such differences as may exist between the species, and, as a result, the degree of difference in the actual requirements of the species for optimum growing conditions. This second object can hardly be attained in the field without most extensive long-term study, and necessarily resolves itself into experiments under controlled conditions of temperature, light, moisture, etc. The present paper deals largely with the results of such experiments.

3. To describe the conditions of the various forest types of the region in such a manner as will explain most clearly the reasons for the success or failure of artificial forestation, so far as these may result from the environment, and the conditions and means necessary for successful natural regeneration after fires or cutting. Here the object is to lead away from empiric silvicultural "systems" and toward the attainment of definite environmental conditions in all silvicultural practice.

4. To convey a conception of the conditions under which the Rocky Mountain forests exist—that is, a view of the climatic and soil conditions of the forest region as a whole.

It is needless to state that not all of these objects have been attained in the present work, which should be considered as something of a pioneer effort, merely blazing the way for much greater efforts and more refined methods which are necessarily for the future to bring forth. The hope may be justified, however, that the results and conclusions of this study will add somewhat to the information on the general subject and encourage the doing of more intensive work.

## SCOPE OF WORK

The entire work, of which this paper represents only a part, is mainly a study of the physical environments of a number of different forest types in Colorado and Wyoming. The detailed study of the composition of these several types has not been given a great deal of attention. Since the forest conditions are common ones and are frequently encountered in the region, it is believed that the indications of prevailing composition and of changes in composition, as they may be expressed in general terms, will be as valuable for present purposes as tabulated statements of the number, size, and kind of trees found at the several stations. The observations of meteorological conditions and of certain soil conditions thought to be fully as important, have, therefore, comprised the major part of the field work.

However, the early efforts (*x*)<sup>a</sup> to summarize and compare these site conditions, as given in meteorological and soil records, and to deduce

<sup>a</sup> Reference is made by number (*italic*) to "Literature cited," p. 163-164.

therefrom the full reasons for the presence or absence of a given species on the various sites were so far from satisfactory that the need for controlled experiments to indicate the comparative reactions of the several species has long been apparent. The amount accomplished in this line is not insignificant, and, since the results aid very materially in interpreting the field conditions, we have chosen to present, first, all of the physiological data that are available. In other words, we shall first consider the manner in which the several species are affected by more or less controlled conditions in the laboratory or greenhouse; then, perhaps, we may weigh more precisely the importance of each condition encountered in the field, and especially avoid the almost universal and inevitable error of placing reliance upon the measurement of a condition which has not really been measured or expressed in the proper terms.

Of the meteorological records which are used in this report the larger share have been obtained by the Forest Service at the Fremont Experiment Station, near Manitou, Colo., and by the Weather Bureau and Forest Service, cooperating in an intensive project at the Wagon Wheel Gap Experiment Station, near Wagon Wheel Gap, Colo. It has been the aim to conduct the meteorological work at both these stations on a high plane of accuracy, with equipment as complete as was thought necessary. At both places the observations have covered elevations from the station headquarters at about 9,000 feet to timber line at 11,000 or 12,000 feet. In both cases, too, soil moisture and soil temperature data have been secured during most of each period of atmospheric observations.

In addition, the Forest Service has equipped and operated from the Fremont Station three subsidiary stations at which the Weather Bureau had previously started the usual observations of precipitation and air temperature. These stations were manned by forest officers, and a very diligent effort has been made to obtain continuous records for them. The stations in question are at the Monument Nursery, in the yellow pine type near Monument, Colo., at the Foxpark Ranger Station, lodgepole pine type, at Foxpark, Wyo., and in the Nebraska sand hills, where the planting of yellow pine and jack pine has reached such large and successful proportions. The first station is only about 20 miles north of the Fremont Station and for all practical purposes may be considered as belonging in the Pikes Peak altitudinal series.

Although the Weather Bureau records of precipitation and air temperature, secured through cooperative observers, were available for a large number of stations in the region covered by this report, and many such stations are within the mountain forest zone, only limited use has been made of such records, and for the following reasons:

(a) Many such observation points are situated, with the small towns, in deep valleys where the conditions met with, especially those of air drainage and soil composition, are not at all the conditions of the adjacent forested slopes. In fact, nearly all such valleys, especially if they possess a distinct flood plain, are devoid of forest cover, and to use their weather records in this study might lead to very erroneous conceptions though the temperature conditions alone are probably not responsible for the absence of forests.

(b) The records obtained at all such mountain stations are only those of precipitation and air temperature. No soil data whatever are secured.

The special work in this project dates from January 1, 1910, when the control station at Fremont was equipped. Records obtained up to 1918

have, in general, been employed, the summarizing of data having been started at that time. However, some records particularly needed have been added since 1918. The period of operation, as well as the equipment of the several other stations, is given under the respective descriptions.

#### METHOD OF STUDY

As has already been indicated, the primary data to be presented in this study are records of climatic and soil conditions in different forest types, and the main object of such presentations must be to show that differences in climatic and soil conditions, between the forest types, either do or do not exist in sufficient degree to account for the varying phenomena of occurrence and growth.

Since the special data collected by the Forest Service in no case cover periods long enough to establish the "normal" conditions of any of the forest types (and by this we mean the average conditions for a period of at least 10 years), and since even "normal" conditions as established by 10 years of observation are certain always to be changed by the addition of further records, it is necessary that we adopt short-period records as a basis for comparison. Such adoption can not be fraught with any serious dangers when the forest types to be compared are in the same general locality, that is, in the path of the same air currents; storm centers, etc. Any considerable separation of the stations, however, especially in a rugged mountainous region, is likely to introduce temporary variations in certain conditions which are not "normal," and particularly in those factors which are most directly influenced by the paths of storm centers. Thus, at the moment of writing this statement, it appears that the storm centers have for some time been passing considerably to the north of the Pikes Peak region, giving that locality rather unusual westerly winds and leaving it with a dearth of moisture, so that at the end of May an unprecedented shortage of water exists. Scarcely 100 miles to the north, unusually large accumulations of snow are reported at the same moment. Again, the moisture factor is most variably influenced by the restricted character of many of the summer showers; especially are the heaviest downpours in a given locality likely to affect only a very small area.

Temperatures are generally not so directly affected by local conditions. Thus the month of December, 1917, was not only an unusually warm month at the Fremont Station but showed the same character over a large part of the western United States, and January, 1918, was, likewise, generally cold to an unusual degree.

We may, therefore, feel safe in comparing the records of any two near-by stations for short periods, whatever the factor under consideration, and we shall demand an increasing period as the distance between stations increases.

Fortunately, the dozen or more stations located in the vicinity of Fremont all come under the same general influences. This is true of the entire area from the plains to the summit of Pikes Peak, with the exception that summer rains frequently fall in one part of the area without wetting other parts. Winter snows may also be so localized, but usually in conformity with altitudinal zones. It is true, two stations not 100 yards apart may on a given day have temperatures varying by a couple of degrees in one direction and on the next day varying in the



opposite direction. Such variations from a consistent relation are, however, always small, and there is every reason to believe that the means of a single month usually express essentially the normal temperature relation between two stations for that month of any year.

Therefore the method of study and the method of presenting results is that of comparing each factor for any station with the corresponding factor at the control station for whatever period observations may have been taken at the outlying station. As a basis for this comparison we have the record of each factor at the control station, measured with practically no variation in method from January, 1910, to date. Exception should be made here to the measurement of evaporation, in which a satisfactory method was not attained until 1917.

The detailed methods of taking meteorological observations, so far as they vary from the standard methods of climatologists the world over, will be described in connection with each condition measured.

#### REVIEW OF OTHER WORK ALONG SIMILAR LINES

Although much systematic ecological study has been attempted in the United States and other countries, and the western portion of our own country has offered an especially attractive field for ecologists because of the sharp contrasts in vegetation and causative factors which are found in relatively small areas, still the main field with which foresters are concerned has barely been scratched. Several studies, which might have been very productive, scarcely satisfy the forester's requirements because of the lack of long-term records.

Be that as it may, a number of authors have obtained facts and deduced conclusions as to the distribution of our Central Rocky Mountain forest trees which we can not afford to overlook. No attempt will be made at this stage to introduce these facts, which may better be mentioned in connection with my own discussions and conclusions. I shall merely list here the works which have a direct bearing on the major problems, and with no attempt to cover the general or specific physiological studies.

Clements (7, 8, 10) in three of his works presents many valuable ideas regarding the relation of Rocky Mountain vegetation to environmental conditions, and with particular reference to the Pikes Peak region, in which much of his investigation has been conducted. The latest of Clements's books, "Plant Succession" (10), published in 1916, may be said to cover the entire ground of the earlier works, bringing all of his observations under one comprehensive theme, namely, the changes which occur in the character of the vegetation of a given area as the result of reactions of the plant forms upon the environment and of gradual changes in the climatic and edaphic (soil) conditions.

In a more specific work Clements (9) gives to foresters a much more concrete idea of the requirements of an important tree species, lodgepole pine, with lesser data on its common associates.

Ramaley (16, 17), working near Boulder, Colo., and in the deeper mountains at Boulder Park, has likewise made numerous observations on the forest types and zones of Colorado. His papers, however, make no claim of extensive systematic measurement of physical factors and hence can be considered as having only suggestive value in connection with the present work. In both of the papers cited the theme is a classi-

fication of Colorado forests according to moisture conditions and composition.

Shreve (24, 25), working at the Desert Laboratory at Tucson, Ariz., and in the Santa Catalina Mountains adjacent thereto, has in a number of papers published results directly bearing on the subject in hand. "The Vegetation of a Desert Mountain Range" is the most comprehensive of these papers and covers most fully the ultimate problems, beyond pure climatology, with which the forester is concerned. Although the Santa Catalinas are somewhat different from the Central Rockies in being surrounded by desert on nearly all sides and in having a different seasonal distribution of rainfall, yet it is apparent that the limiting factors for the occurrence of a given species must be essentially the same in the two regions; else all attempts to formulate a systematic ecology would be vain. These factors may not, it is true, appear quantitatively alike under present methods of measurement, but, if so, we should seriously question the method of measurement.

It will be noted that in the papers referred to Shreve ascribes the main control of the upward extension of desert plants to temperature, and in another paper (23) he has quite convincingly shown that the duration of freezing temperatures is all-important with plants accustomed to the ordinarily warm winter air of the desert. With this view we shall have no reason to take issue. The other main conclusion, that lack of moisture limits the downward extension of the forest species, individually and collectively, will, it is believed, be found subject to question or at least modification.

Robbins (18) has prepared the most recent and complete summary of Colorado's climatic conditions in relation to native vegetation and agriculture. While, as stated by the writer, this work attempts to show only a qualitative relation between climate and plants, it is, nevertheless, excellent both in the data systematically presented and in the relations described. For the most part these relations are too broadly stated to be of direct assistance in the present study. A quotation from the discussion of the freezing of plants is of considerable technical interest:

It is a familiar observation that some of the more tender plants are injured by temperatures above the freezing point; and that, on the other hand, there are many plants that may withstand temperatures considerably below the freezing point. This statement may apply not only to dormant plant parts, but to swelling buds, open flowers, and forming fruit as well. The plants at timber line and above are subject to freezing temperatures almost every night in the year. The exact nature of this immunity to low temperatures is not known.

Weaver (27) in 1917 studied the desert-to-mountain formations of Washington in a manner not unlike Shreve's, and ascribes the changes in vegetation mainly to increasing soil moisture and decreasing evaporation with a rise in elevation.

Shantz (20, 21) has dealt with problems intimately connected with the factors limiting the downward extension of the Rocky Mountain forests. Particularly is Shantz's work enlightening to foresters in the thorough treatment of the soils problem. He has made it plain that the lighter soils of the plains, characterized by bunch grass, show much less variation in productivity from year to year than the heavier, loamy soils which develop the grama-buffalo-grass association. This difference is due to greater penetration of both moisture and roots in the lighter soil as well as to the greater availability of the moisture when the content becomes low, tending to encourage slow growth, and the longer lived bunch grass.

By analogy we may say that the same relation exists between grassland or sage brush and the lowest type of forest, commonly called "woodland" by foresters, since it is obvious that there can be no important change in climatic conditions in the small space between the centers of development, and that the development of the forest is made possible by the slight soil changes resulting from elevation, surface erosion, and leaching, all of which maintain a younger soil.

The establishment of the forest experiment stations in the western United States, beginning with that at Flagstaff, Ariz., in 1908, gave unparalleled opportunity for the collection of forest and climatological data over a period of years. As a result, studies similar to the present one have been initiated in Arizona, California, and Idaho, and the study of forest and herbaceous vegetation has been carried on in connection with a number of experiments at the Utah Station, where grazing problems occupy the attention first. The strictly forest studies, however, are for the most part not yet ready for publication.

Pearson (14) at the Flagstaff Station early investigated the effect of yellow pine forests upon local climatic conditions, by securing data in the forest, the edge of the forest, and "parks" (grassy, meadowlike openings) of considerable extent. While the climatic conditions recorded by Pearson are interestingly compared with our own, this study can not be said to throw very much light on the conditions governing different forest types.

However, Pearson (15) has recently made available the results of observations at a series of stations in the San Francisco Mountains, in a very comprehensive way, and we shall have reason frequently to compare his conditions with our own.

We have, similarly, had access to an unpublished report by Larsen<sup>4</sup> on the conditions of Montana and Idaho, which has been extremely helpful in giving comparable data.

The problem of the prairies in the Middle West, and their physical relation to the occasional forested areas, has received considerable attention, and this problem is not too remote from our own entirely to lack interest. On this subject may be considered the work of Shimek (22), who concludes, regarding Iowa conditions:

1. Exposure to evaporation as determined by temperature, wind, and topography is the primary cause of the treelessness of the prairies.

and

3. Rainfall and drainage, while of importance because determining the available supply of water in both soil and air, are not a general, determining cause, both frequently being equal on contiguous forested and prairie areas.

Shimek also dismisses fires as a cause of the absence of forests. It is believed that the later conclusions of Weaver and Thiel (26, 28), with reference to Minnesota, are essentially in agreement with this. The point which seems to have been overlooked here, and in all similar discussions, is that forests occur usually on the slopes of ravines or on hillsides, where the old soil is being rejuvenated by a secondary erosion and where, even with less moisture than in the heaviest soils, the availability may be greater. Considered from this angle, the occurrence of forests in the prairie region is exactly parallel to their occurrence on the first mountain elevations at the edge of the plains.

<sup>4</sup> LARSEN, J. A. CLIMATIC STUDY OF FOREST TYPES, DISTRICT 1. U. S. Dept. Agr. Forest Serv., unpublished report, 1918.

It will be fairly evident from the reading of the few treatises which have been mentioned that the region under discussion has not been neglected by ecologists. It will be equally evident that there is still room for much systematic effort in the study of the environmental factors in order that the theories advanced regarding the distribution of mountain forests may be more thoroughly tested by well-established facts. The most apparent fact, after considering all of these regional studies, is that so far ecology has given the physics of the soil-moisture problem entirely inadequate attention.

## II. PHYSIOLOGICAL STUDIES LEADING TO AN INTERPRETATION OF THE ENVIRONMENTAL DATA

It is quite generally recognized that the result of studying any condition in nature, even when the method of study is strictly quantitative, should not consist wholly in presenting the accumulated facts but quite as much in placing a logical interpretation on those facts. In the present study we are dealing not only with a great variety of natural conditions which require quantitative expression but with a variety of growing entities whose behavior and reaction to known conditions can not be determined by casual observation. For example, the mere fact of finding a spruce tree growing at the water's edge does not prove that the tree uses or requires an unusual amount of water, much less that it is growing in that particular spot primarily because of the moisture, or even indirectly because of the moisture. It would be as logical to say that because the alligator spends a good deal of his time in the water, he must drink and must require for physiological processes an extraordinary amount of water. This may not be true at all; he may be a most abstemious animal.

The point is that in ecology we dare not take the conditions of growth as *prima facie* evidence of the requirements of growth, even though it be true that none of the conditions can be altered without affecting the character of the growth. This is especially true when we are compelled, as in the present instance and in most ecological studies so far made, to speak of requirements in a relative rather than an absolute sense, that is, when we are simply trying to compare the requirements of several species rather than determine them absolutely for any species. This may be illustrated by a point which has appeared very forcefully in the present study. Taking the superficial appearance of soil conditions as a measure of relative requirements, foresters have repeatedly stated that the moisture requirements of spruce were greater than those of yellow pine. Now, there could be no objection to saying, and probably no error in saying, that spruce requires or at least develops best in a fresh, moist soil of high water-holding capacity. This would be an absolute expression which would simply gain in accuracy as the soil conditions were further analyzed. We might infer, and would be likely to do so, because of the character of the soil occupied, that spruce must use a great deal of water in its development. Such an inference would be unwarranted, but would be especially dangerous if we should say, comparatively, that spruce uses more water than pine. Here we are treading on absolutely unsafe ground. On the face of it there is no scientific basis for such a statement, if we use simply the evidence of the field conditions observed. And even if this were true of the spruce forest in the aggregate, that bespeaks nothing as to the individual.

It has been, therefore, in the hope of partially overcoming the inherent weakness of a comparative field study that certain observations have been made under laboratory conditions, permitting a better knowledge of the trees themselves, hence a safer interpretation of the environmental conditions which surround them in the field, and, perhaps, a clearer conception of how those conditions should be measured in the future to express a logical relation between the environment and growth.

For the most part these special observations have been made upon the four important species which are involved in the field study, namely, western yellow pine, Douglas fir, lodgepole pine, and Engelmann spruce. Two other species, forest "weeds," have been studied to some extent, namely, limber and bristlecone pines (*Pinus flexilis* and *P. aristata*). A few observations have also been made on the Lake States pines and other conifers not indigenous to the Rocky Mountains.

In the interest of brevity, some details of the conditions of these experiments may have been omitted which might be considered as having important bearings. Anyone wishing to investigate these details will be given all possible assistance.

#### TRANSPIRATION TESTS IN 1917

To establish the water requirements of some of the Rocky Mountain trees in the same terms as used by Briggs and Shantz (6) for agricultural crops, and to determine the relative transpiration rates of the species as a basis for gauging their moisture requirements in the field, transpiration tests were conducted in the greenhouse of the Fremont Experiment Station for a period of about six months in 1917. The experiment was repeated in 1920.

It should be recognized at the outset that the greenhouse did not present natural conditions for the growth of any of the species, the air temperatures being higher than commonly occur except possibly in the lowest zone of the region, and the air movement considerably less than the wind which would occur in any situation out of doors. For these reasons, though we may speak of the "absolute water requirements" of the trees in this particular test, these requirements are not an indication of what the water use might be under any other conditions; and it would be best, as Briggs and Shantz have done, to assume only that we have established relative requirements of the several species for one set of conditions. These relations may or may not hold good under other conditions. Briggs and Shantz found that relative water requirements of different species did not vary much under different conditions, though the absolute requirements of all might be twice as great during a dry season as during a moister one. Thom and Holtz (26) found that the physical conditions might vary sufficiently to change even the relative requirements of different species, but their more important result was to show that the absolute water requirement increases with the availability of moisture.

It would appear that the high temperatures and low wind velocities occurring during these tests should tend to stimulate assimilation rather more than transpiration, so that the absolute water requirements here would be less than under normal field conditions for any one of the species. This, however, may not be true. For this and other reasons appearing later it is difficult to compare the absolute requirements with those of agricultural crops.

## MATERIAL STUDIED

As the means were not at hand for treating large trees in the intensive manner required in such a study, efforts were confined to nursery specimens, 5 and 6 years old at the outset. These had all been developed in the nursery of the Fremont Station, with practically uniform soil conditions and with no artificial watering except as small seedlings, so that all should have been in much the same condition at the outset.

Two specimens each of yellow pine, Douglas fir, lodgepole pine, Engelmann spruce, limber pine, and bristlecone pine were taken for potting, while a third specimen of each was taken at the same time for drying, in order that the initial dry weight of the specimens to be grown might be computed. This drying and all other dryings required in these studies were done in hot water bath or controlled hot air oven at a temperature of about 92° C. and without vacuo.

To determine the initial green weights, and also the green weights at the close of the test period, each tree was washed to remove adhering soil particles, whipped vigorously through the air to remove free water, and placed immediately on the scales. After this the potting was accomplished as soon as possible.

The initial weights varied from 7 to 14 gm. and heights from 3 to 6 inches, the spruce being, on the whole, best developed for its age. No measurements other than weights were taken at the outset. At the end of the test period the green weights were taken; each tree was photographed to scale, as shown in Plates 1 to 3; measurements were made to determine the mean needle dimensions of each tree and the ratio of surface to volume (the whole volume having been determined by immersing the top in water to the root collar); finally the remains were oven dried, and later the dry material was reduced to ash in a porcelain dish over a Bunsen flame.

From the volume displacement and needle dimensions we are enabled to compute the area of leaf surface in each case, with a very considerable but general error on account of the stem volume included. This will, at least, give some basis for comparison with other experiments in which the leaf surface is the basis for calculations of water loss. Because of the great inaccuracies involved in the method and the practical impossibility of applying it to a large tree, and also because it is believed that transpiration is so largely controlled by the area exposed to insolation and the consequent total absorption of radiant energy, we have also used another basis for expressing leaf area, which we shall call "leaf exposure." This is obtained from the tree photographs, which are against a background of cross-section paper, by estimating the proportion of each square inch which is obscured by the foliage. This method, if carefully followed, gives reasonably consistent results, except in cases like tree No. 8, in which the focus is bad.

It is seen that the "leaf exposure" could not be more than one-third of the whole leaf surface, and owing to a great deal of overlapping of needles, as well as elimination of stem, the data in this case compare generally on a basis of about 1 to 6. But with the limber and bristlecone pines, whose foliage is very compact, the ratio is more nearly 1 to 10.

## SOIL

For potting, open-topped galvanized cans were used, 4 inches in diameter and 10 inches deep. No drainage openings were made in the

cans; but, to encourage aeration of the soil, a 2-inch florist's pot was inverted in the bottom of each can, and a glass tube one-eighth inch in diameter was so bent and placed that its lower end opened into the pot and its upper end just above the rim of the can. This tube served for supplying the necessary water and was at all times left open for aeration of the soil. It is believed that the amount of vaporization through the tubes was insignificant, though no control tests were made at the time. We are enabled to approximate the loss from this source by the observations in 1920. However, in 1917, the soils were never allowed to become greatly heated, the potting cans being placed in similar cans having diameters of 6 inches, so that the sun never shone on the lower portions of the pots.

Before weighing and potting, each tree was trimmed so that the longest roots would not be cramped in the can. The longer roots were spread around the porous pot in the bottom of the can, and the others were placed as the pots were filled, so as to be evenly distributed throughout the soil. When the cans were all filled to within a half inch of the tops, they were sealed with a 2 to 1 mixture of paraffin and vaseline, which held very well throughout the season in spite of occasional melting.

The soil used was a specially prepared loamy sand of granitic origin, containing considerable humus derived mostly from leaves of limber pine and *Arctostaphylos* sp. Both sand and humus were sifted through one-eighth-inch screen. The resultant mixture was what would ordinarily be considered a good potting soil. It was thought to be desirable to insure an abundance of nutrient material, and there is no reason for supposing that this was overdone.

None of the soil placed in the pots was oven-dried, but a weighed amount of air-dried soil was used in each, and during the process of potting several samples were taken for the purpose of determining the moisture content. The net oven-dried weight for each pot was then computed.

The saturation capacity of the soil used was originally determined to be about 40 per cent, and, in accordance with Kiesselbach's (12) finding that transpiration occurs most freely when the soil is about half saturated and the theory of Hilgard (11) that half saturation permits the desired aeration, 20 per cent moisture was adopted as the standard at which the soil would be kept. Later it was found that with greater compactness this saturation might be much less, and after centrifuging, as low as 25.8 per cent. However, the figure 31.9 probably applies most nearly to the condition of the soil in the pots. The corresponding capillarity was 28.2 per cent, and the moisture equivalent at 100 gravity was 11.05 per cent, using the term in the same sense as it is used by Briggs and Shantz (5) for the water-holding capacity under a force of 1000 gravity. The mean wilting coefficient was determined in 1920 to be 3.47 per cent for Douglas fir and 3.91 per cent for spruce, or an average value of 3.69 per cent. On this basis, and assuming that the 20 per cent maximum moisture was evenly distributed, we should have as the availability  $\frac{20 - 3.69}{20} = 0.816$ . It is more probable that the moisture within

reach of the roots, at the bottom of each pot, was 25 per cent or more, making the availability at least 0.850.

Table I shows all of the fundamental data regarding the trees, the amounts of soil used, and the gross weight of the pots as they were maintained throughout the season.

TABLE I.—Description and condition of trees used in transpiration measurements

Species.....	Yellow pine.		Douglas fir.		Lodgepole pine.		Engelmann spruce.		Limber pine.		Bristlecone pine.	
	1	2	3	4	5	6	7	8	9	10	11	12
Can No.....	5	5	5	5	5	5	5	5	6	6	6	6
Age of trees (years).....												
Initial green weight (gm.).....	10.55	13.48	6.77	8.90	7.52	8.80	14.38	13.46	8.10	14.30	10.85	10.15
Green dry factor of sample tree.....	2.45	2.45	2.56	2.56	2.51	2.51	2.33	2.32	2.37	2.37	2.41	2.41
Initial dry weight (computed).....	4.31	5.50	2.65	3.48	3.00	3.51	6.20	5.80	3.44	6.12	4.30	4.31
Final weight.....	16.97	20.56	10.05	16.80	13.28	15.20	26.06	28.20	12.15	16.84	15.39	14.06
Dry.....	5.96	7.46	4.28	5.85	4.31	5.24	10.41	10.36	4.48	10.48	5.95	5.49
Weight gain for season.....												
Green.....	6.42	7.08	3.28	7.60	5.76	6.40	11.68	11.04	4.05	2.32	4.54	4.11
Dry.....	1.15	1.96	6.16	2.37	1.13	1.73	4.21	4.78	1.06	0.35	1.45	1.38
Abs.:.....												
Weight (gm.).....	.31	.30	.20	.49	.25	.34	.50	.64	.20	.33	.39	.35
Percentage of final green weight.....	1.83	1.46	1.99	2.97	1.88	2.24	1.92	2.25	2.39	1.96	2.53	2.45
Computed leaf surface.....												
Volume of top by displacement (cc.).....	8.8	11.9	7.4	10.3	7.0	8.7	21.1	19.1	8.5	9.3	9.7	8.8
Length of needles (cm.).....	7.23	9.87	2.20	1.90	4.90	5.58	1.83	1.53	3.00	3.37	3.07	3.02
Length of needles (mm.).....	1.13	1.30	2.3	1.10	1.12	1.93	.90	.77	.92	.90	.80	.73
Cross section (mm.).....	X	X	X	X	X	X	X	X	X	X	X	X
Ratio of area (in sq. mm.) to volume (in cu. mm.).....	55	58	44	69	43	45	70	48	75	75	75	53
Leaf exposures (sq. cm.).....	459	531	497	713	426	626	1,072	1,293	563	643	713	770
Leaf exposures (sq. cm.) from photographs.....	64	79	64	100	56	66	153	149	66	71	71	71
Net dry-soil weight (gm.).....	2,580	2,540	2,508	2,516	2,479	2,562	2,380	2,308	2,566	2,476	2,405	2,405
20 per cent water (gm.).....	516	510	508	503	495	513	476	479	513	501	496	481
Tare (gm.).....	1,002	980	996	1,001	973	988	1,037	1,012	1,015	977	1,017	1,009
Gross, with tree as above.....	4,128	4,054	4,051	4,029	3,955	4,072	3,907	3,902	4,102	3,994	4,000	3,905

a At end of tests, all root tips are old and lignified.

b Root tips very short, showing slow or late development.

c Cross sections of yellow pine and lodgepole pine computed as semicircles; limber pine and bristlecone pine as fifth circles, more or less; Douglas fir as ellipses; Engelmann spruce as rectangles.



## PROCEDURE

The trees were taken from the nursery and potted about April 15, 1917. From that time until April 26 they were kept in a warm room, without sunlight, to encourage root growth and establishment in the soil. On April 26 the cans were first brought to standard moisture content, and measurements of transpiration losses were begun. From that date to June 3 they were kept in the window of a warm room, where they received light for only a few hours each day. The pots were frequently but not regularly shifted in position.

On June 3 they were placed on a revolving table in the greenhouse, where they remained until the close of the test on November 14, with the exception of one day out of doors. This table was handled in several ways, the power available for rotating it at the outset being inadequate. At first a small motor was used, the motor being cut in each minute for a period of a second or more, so as to give the table a fraction of a revolution. For several short periods the table was turned by hand, a quarter revolution about once each hour. A water motor was finally used, which for a time kept the table revolving continuously. This, however, seemed to have a theoretically objectionable feature in that the trees were constantly passing from light to shadow, in a very unnatural manner. The driving belt was therefore arranged so as to move the table a peripheral distance of about 6 inches each minute, or, say, a complete revolution in about 25 minutes.

The pots occupied the periphery of the 4-foot table, various types of evaporimeters being placed between them. Within this circle was placed an air-and-soil thermograph, the arm of the air register being shaded, while the soil bulb was blackened and so placed, with its long axis horizontal, as to receive as much sunlight as the trees. With the assistance of maximum and minimum registering air thermometers and a thermometer attached to the blackened bulb of the soil thermograph, there were thus recorded both air temperatures and "sun" temperatures. In addition, a psychrometer was used during the morning observation each day, giving a rough indication of prevailing vapor pressures.

The most important question of procedure, of course, concerns the method of determining water losses. As shown by Table I, each pot had, at the outset, a known gross weight when its soil contained 20 per cent of moisture. The aim was to keep the moisture to this standard by replacing losses each day. It was only necessary to determine the amount of water required to bring the pot up to standard weight in order to record the loss for the preceding period. This was accomplished by placing the pot on one pan of the scale, the standard weights on the other pan, and filling from a titrating burette until a balance was reached. The amount drawn from the burette was, therefore, the measure of the loss. The measurements of transpiration, it is thus seen, were actually volumetric, even though scales were used. This introduced no error worth considering, as the temperatures at the observation hours varied scarcely at all from about 50° F.

The burette was graduated to 0.1 cc. The scales were barely sensitive to 0.1 gm. under the usual load of 4,000 gm. However, errors from this source should be compensating. Whenever the filling was carried too far, as not infrequently happened, the overload was determined and allowed for, and also carried to the record for the next period.

All observations were made in the early morning and before sunlight had reached the trees, and when, therefore, the transpiration rate would be almost at its minimum. The order of measurements was invariable, and the time rarely varied more than 15 minutes from the standard.

Now, in fact, though it has been stated that the plan was to maintain standard moisture in each can, it is readily seen that the moisture was most of the time below standard. The extent of the ordinary depressions was very small. The largest single loss between fillings, 157.1 gm., would mean a moisture content at the end of the period of 13.9 per cent, or a depression of 6.1 per cent. The average periodic loss of the heaviest water-user was 23.76 gm., and the average depression below standard moisture, therefore, only 0.93 per cent. This average depression would reduce the availability of the moisture only from the approximated value 0.852 to 0.846.

On the other hand, the distribution of the moisture from top to bottom of each pot, as shown by examination at the end of the tests, was not all that might be desired. The lowest inch of soil was practically saturated, and above this the moisture decreased so that just below the paraffin the soil was only freshly moist. In spite of this, rootlets had penetrated to all sections of the soil. It seems evident that, except with the most extreme depletion noted, there was probably within reach of the longest roots at all times practically saturated soil.

The atmospheric conditions of the greenhouse, as has been stated, were not such as would occur naturally in any of the sites where these species grow. At times the ventilators were kept closed and the air temperatures were allowed to go as high as they would with full sunlight. At other times the ventilators were opened and all possible draft was developed; and, of course, under these conditions the air of the greenhouse did not become so warm. Again, sunlight was excluded on certain days to see what effect this would have on transpiration rates. On two or three occasions when cloudy weather prevailed, an interval of 2 or 3 days was allowed to elapse between measurements, since the losses were very small. On two occasions when the writer was not there to make the measurements, the intervals were considerably longer, the trees being shaded by canvas for the entire period.

No apparent injury resulted from the high temperatures in the greenhouse, except to tree No. 4, Douglas fir. On June 24, which was a clear day of exceptionally low humidity and high evaporation rate, two of the newly formed shoots on this tree wilted and did not recover. Nevertheless the tree continued to function properly. The other Douglas fir (No. 3) followed No. 4 very closely, but after the first of September showed a gradual decrease in its response to transpiration stimuli and when unpotted was found to be deficient in new root growth. In the normal trees it appeared that many of the root tips had continued growth to the end of the season, while in this one growth had evidently ceased much earlier.

Tree No. 10, limber pine, at the end of the season showed very short growing tips on the roots, indicating that root development had been very sluggish or had started very late. This sluggishness is doubtless related to the small weight accretion.

## RESULTS

The amount of transpiration for each tree during each period of the study from April 26 to November 14 has been tabulated, and it is found that the tree-to-tree relations hold very closely, from day to day, in spite of great variations in the environmental conditions. It is not relevant to present purposes to present the detailed data. In Table II the transpiration by months is given and the tree performances are summarized. In correcting for the loss of water directly from the soil, the amount of 107 gm. for the season has been arrived at by considering the daily loss the same as in 1920. While in 1917 the pots were more fully protected from the sun, yet considerably higher air temperatures were attained, so that this allowance seems justified and can hardly be enough in error appreciably to affect the results.

TABLE II.—Summary of transpiration in the test of 1917

Species.....	Yellow pine.		Douglas fir.		Lodgepole pine.		Engelmann spruce.		Limber pine.		Bristlecone pine.		All.
Tree No.....	1	2	3	4	5	6	7	8	9	10	11	12	
Transpiration by periods:													
April and May—													
Grams.....	42.1	187.5	143.3	157.1	189.9	221.3	223.6	223.3	86.3	86.7	137.5	136.6	1,835.2
Per cent.....	2.3	10.2	7.8	8.6	10.4	12.0	12.2	12.2	4.7	4.7	7.5	7.4	.....
June—													
Grams.....	360.8	704.2	337.2	348.8	349.7	411.0	433.5	535.1	265.7	215.2	322.4	388.5	4,654.1
Per cent.....	7.8	15.2	7.2	7.5	7.1	8.8	9.3	11.5	5.7	4.6	6.9	8.4	.....
July—													
Grams.....	285.1	427.9	221.5	235.3	182.9	208.9	212.5	354.9	173.5	132.5	175.8	213.6	2,843.5
Per cent.....	10.0	15.0	7.8	8.3	6.4	7.3	8.2	12.5	6.1	4.7	6.2	7.5	.....
August—													
Grams.....	388.4	571.6	293.0	321.8	256.7	266.3	302.6	581.0	244.6	203.1	266.9	297.7	4,025.7
Per cent.....	9.7	14.2	7.3	8.0	6.4	7.4	7.5	14.4	6.1	5.0	6.6	7.4	.....
September—													
Grams.....	402.1	515.4	155.8	334.1	208.9	238.9	342.9	578.1	234.0	189.1	260.6	268.7	3,728.6
Per cent.....	10.7	13.8	4.2	5.0	5.6	6.4	9.2	15.5	6.3	5.1	7.0	7.2	.....
October—													
Grams.....	382.6	479.5	44.9	260.2	176.1	202.4	303.3	496.4	215.5	171.7	266.8	249.9	3,259.3
Per cent.....	11.8	14.7	1.4	8.0	5.4	6.2	9.3	15.5	6.6	5.3	8.2	7.6	.....
November—													
Grams.....	201.4	250.3	10.2	89.2	85.8	92.7	134.2	221.1	100.3	72.5	126.3	115.9	1,499.9
Per cent.....	13.4	16.7	.7	6.0	5.7	6.2	8.9	14.8	6.7	4.8	8.4	7.7	.....
Total, April to November													
(grams).....	2,062.5	3,136.4	1,205.9	1,748.5	1,430.0	1,671.5	1,972.6	2,999.0	1,310.9	1,070.8	1,556.3	1,670.9	21,844.3
Correction for direct evaporation.....	107	107	107	107	107	107	107	107	107	107	107	107	1,284
Net loss through leaves.....	1,955.5	3,029.4	1,098.9	1,641.5	1,323.0	1,564.5	1,865.6	2,892.0	1,213.9	963.9	1,449.3	1,563.9	20,560.3
Transpiration—													
Grams per gram weight accretion—													
Green.....	308	428	335	216	210	244	160	192	300	415	319	381	263
Dry.....	1,695	1,545	615	693	1,002	995	444	666	1,100	2,750	990	1,231	880
Grams per square mean weight.....	142	178	131	129	127	130	92	138	100	62	110	128	123
Grams per square centimeter leaf surface.....	4.26	5.7	2.21	2.3	3.11	2.5	1.74	2.24	2.16	1.5	2.03	2.03	2.48
Grams per square centimeter leaf exposure.....	30.6	38.4	17.2	16.4	23.6	23.7	12.2	19.4	21.3	14.6	20.4	22.0	20.6

\* Percentage of the transpiration of all the trees.

## Water Requirements

This term, as used by Briggs and Shantz (6), denotes the units of water used by a plant for the production of a unit of dry plant material.

The first computation in the lower section of Table II gives corresponding data for the small trees whose transpiration has been measured. Averaging the results for each pair of specimens, we find the species arranging themselves according to Table III. In this table the use per unit of green weight is also given, since the green-weight accretions were directly measured, whereas the original dry weights were obtained indirectly, as explained.

TABLE III.—*Water requirements*<sup>a</sup>

Species.	Water used per unit of—	
	Dry-weight accretion.	Green-weight accretion.
	Gm	Gm.
Limber pine.....	1,947 (678)	358 (49)
Yellow pine.....	1,555 (8)	366 (52)
Bristlecone pine.....	1,110 (94)	350 (26)
Lodgepole.....	954 (41)	237 (6)
Douglas fir.....	684 (8)	276 (50)
Engelmann spruce.....	525 (68)	176 (14)

<sup>a</sup> The probable error in the average of two figures is indicated by the quantity in parentheses.

Considering only the first column of figures, it is seen that the probable error in the averages is large in two or three cases, and especially so with limber pine, so that this species might possibly belong after yellow pine in the list. In fact, considering that it is the specimen of high water requirement (Tree 10, 2,750) which showed at the end of the season evidence of lack of vigor, it seems altogether probable that the normal or true water requirement of limber pine should be gauged by the lower figure. Also, from the fact that No. 10 was at the beginning a larger, probably more succulent specimen, we may quite confidently place this species on a par with bristlecone pine.

No other change in the order of arrangement is indicated as probable by the variations in the first column. However, examining the second column of the table, it is seen that the requirement of Douglas fir is greater than that of lodgepole. But, again, it is the Douglas fir specimen of higher water use (No. 3, 335) which behaved abnormally, its activity apparently almost ceasing before the end of the season, so that we must incline toward leaving Douglas fir in the position indicated by the first column of figures.

We shall not attempt here to discuss the cause of these variations, though that, too, is most interesting and will be at least partially clarified later.

It must be recognized that the relative water requirements, or ability to make growth with a given volume of water, while having a direct bearing on the relations of two or more species which compete with each other, may tell very little as to the ability of a tree of a given species to withstand the drought or wind exposure of a given site. The water requirements no doubt explain in some degree the gradual suppression

and crowding out of yellow and limber pines by fir or spruce, the similar elimination of lodgepole when spruce seriously competes with it. The water requirements may explain certain things which we have habitually ascribed to presence of or lack of shade "tolerance." But it does not necessarily mean that yellow pine, for example, might not resist transpiration and survive under rather rigorous conditions where no question of relative growth rate was involved.

#### Resistance to Transpiration

To obtain a better idea of relative resistance to transpiration we should consider the water losses, under equal conditions, as related to plant mass, leaf area, or leaf exposure. In Table IV the data are summarized on each of these bases, but the species are arranged in the order indicated by the relative transpiration per unit of leaf exposure.

TABLE IV.—*Water losses per unit of leaf area and plant mass*

Species	Seasonal water loss (grams)		
	Per square centimeter of leaf		Per gram mean green weight
	Surface	Exposure	
Yellow pine.....	4.98	34.5 (3.)	160
Lodgepole .....	2.80	23.6 (0)	128
Bristlecone .....	2.03	21.2 (0.7)	119
Limber pine .....	1.83	18.0 (2.8)	81
Douglas fir .....	2.26	16.8 (0.3)	130
Engelmann spruce.....	1.99	15.8 (3.0)	115

On the basis of the transpiration per unit of leaf exposure (which is believed to be the safest basis we have) or per unit of mass, the order of arrangement is essentially the same as in Table III. It is, perhaps, significant that the four important forest trees, yellow pine, lodgepole, Douglas fir, and spruce, appear in the same order as in the preceding table, while limber pine and bristlecone pine have moved to positions just below lodgepole. Taking the data at face value, let us consider for a moment what these qualities of limber and bristlecone pine must mean. In the first place, it has been seen that these species, which are admittedly very adaptive "weed" trees, use considerable water without making much growth. In the second place, we see that relative to their leaf area or whole mass they use very little. In other words, they are in some way adapted to protect themselves from water loss, but along with that adaptation, perhaps as a result of it, they have very meager ability for making use of light in photosynthesis. It is readily seen how this may happen. If either of these trees, accustomed to growing on bare sites where there is no competition, has adapted itself through reduction in the number and size of its stomata, moisture loss is reduced and the ingress of carbon dioxid is likewise reduced. If, again, as the writer has sometimes noted, the needles are closely appressed for the purpose of mutual protection, then moisture is saved at the expense of the full insolation of each leaf. The same might be the result of thickened epidermis or heavy palisade tissue. It thus appears that almost any adaptation for the conservation

of water must result in inefficiency in photosynthesis, and it might possibly be stated as an axiom that a weed is a plant which through protective adaptations is facultative for a variety of environmental conditions but which for the same reasons is incapable of making a standard rate of growth.

The impression is, then, obtained that yellow pine, lodgepole, Douglas fir, and spruce are resistant to transpiration in almost the same degree as they are capable of making use of water for their development, and that none of them possess any special adaptations for preventing water losses which interfere with growth. The cause for the differences between the several species should, then, be sought in those internal conditions which may determine their photosynthetic capacities and the behaviors of their cell contents as solutions, as was done by Salmon and Fleming (19) in the study of the winter hardiness of grains.

Before turning to that subject, however, which will be considered under the heading "Sap density," the transpiration rates should be further analyzed.

#### Periodic Transpiration

In Table II the transpiration of the trees by months has been shown, with the amount for each tree expressed as a percentage of that for all the trees. From these data it may be observed that only a few of the trees maintained stable positions with respect to the whole. The greatest significance of this is to indicate that, if the growth could have been measured for shorter periods, the relative water requirements might not have been the same as those for the whole season.

Comparing the transpiration at the beginning and end of the season (April to June against October to November), it is found that the species may be arranged in the following order, those which show the greatest relative increases being placed first: Yellow pine, limber pine, spruce, bristlecone, Douglas, fir, and lodgepole. Lodgepole and Douglas fir show actual decreases.

If we should eliminate trees 3 and 10, which were apparently affected by some unknown factor, it would scarcely change the relations of the species.

These data at least indicate that the internal conditions which control transpiration are variable and probably are affected by the building of new tissue, accumulation and distribution of carbohydrates, and other changes which may occur in a season's growth.

#### Response of Transpiration to Light and Air Movement

The variations of each tree during the season, as shown above, almost preclude the possibility of determining the responses of the species to the different environmental conditions which were produced from day to day, since such comparisons, to use the available data, must include days during all parts of the season.

Taking as a standard for each species the days during the season when there was no ventilation in the greenhouse and when the total recorded sunlight was in excess of 400 minutes per day, it is found, as shown by Table V.

1. That for days having from almost none to 400 minutes of sunlight, with other conditions equal, all the species show about 60 per cent of the transpiration for a standard day.

2. With bright diffuse light, such as might penetrate the canvas curtains on a sunny day, the transpiration varies from 21 per cent of standard for lodgepole to 32 per cent for yellow pine and Douglas fir.

3. With dull diffuse light, as on cloudy days, the percentages vary from 17 per cent to 23 per cent of standard. Though lodgepole shows at all stages the greatest depression from the lack of sunlight, it is hardly safe to say that this is a specific character.

TABLE V.—*Response of various species to different conditions of light and air movement*

Conditions	Number of days	Species					
		Yellow pine.	Douglas fir	Lodgepole.	Engelmann	Limber pine.	Bristlecone pine
Average transpiration (cubic centimeters per day, standard), no ventilation, over 400 minutes sunshine.	12	21.82	13.59	11.86	19.84	9.62	13.22
Proportionate transpiration:							
No ventilation, less than 400 minutes sunshine	11	.62	.62	.54	.62	.62	.59
No ventilation, bright diffuse light	19	.32	.32	.21	.26	.31	.25
No ventilation, dull diffuse light	7	.23	.20	.17	.22	.18	.18
Some ventilation, over 600 minutes sunshine, temperature over 75° F.	4	1.05	1.08	1.35	.98	1.18	1.18
Some ventilation, over 600 minutes sunshine, temperature under 75° F.	2	.91	1.02	1.12	.86	1.11	1.10
Some ventilation, 400 to 600 minutes sunshine, temperature over 75° F.	7	1.27	1.12	1.40	1.08	1.41	1.23
Some ventilation, 400 to 600 minutes sunshine, temperature under 75° F.	13	.09	1.06	1.19	.99	.94	1.08
Some ventilation, less than 400 minutes sunshine, temperature over 70° F.	10	1.00	1.05	1.06	.90	1.00	.98
Some ventilation, less than 400 minutes sunshine, temperature under 70° F.	8	.59	.58	.64	.61	.63	.57
Some ventilation, diffuse light	5	.21	.28	.28	.31	.28	.29

\* Only tree No. 4 used, account seasonal change in No. 3.

4. With ventilation, the transpiration of all species is increased over that without ventilation, other conditions being about the same. The amount of ventilation in the greenhouse was not sufficient to produce striking changes. The exposure of the trees outdoors for one day did not materially increase the transpiration rate, temperatures being considered.

Finally, since the specific responses are subject to the seasonal changes already noted, we may examine the results expressed by the total transpiration of the 12 trees during each day or longer period.

An attempt has been made to relate this to the vapor deficit, or differential between the atmospheric vapor pressure and the saturation pressure conceived to exist within the leaf, as determined by mean temperatures. For the latter there is available either the air thermograph record or the "sun" thermograph (blackened bulb) record. The latter seems preferable in theory, but the record is not very trustworthy because of very large corrections in the instrument as used during most of the season. This, together with the fact that atmospheric vapor pressures were determined only in the early part of each period (9 a. m.) and at its end (7 a. m.), makes the computation of vapor deficits the roughest approximation. In addition, it becomes evident that the leaf temperatures (and saturation pressures) should not be considered as equal to the black-bulb temperatures, but more nearly equal to what the wet bulb of the psychrometer would show synchronously. As the psychrometric data are not sufficient even to approximate the mean wet-bulb temperatures, the results of "sun temperature" computations alone will be shown. Recognizing that transpiration at night, because of the lack of sunlight, is in a different category from that during the day and



should be given much less weight in the total, the general scale of temperatures has been raised by computing the daily means as follows: The 12 hourly temperatures from 7 a. m. to 6 p. m., inclusive, are added to the temperatures at 8 p. m., 12 m. and 4 a. m., and the total is divided by 15.

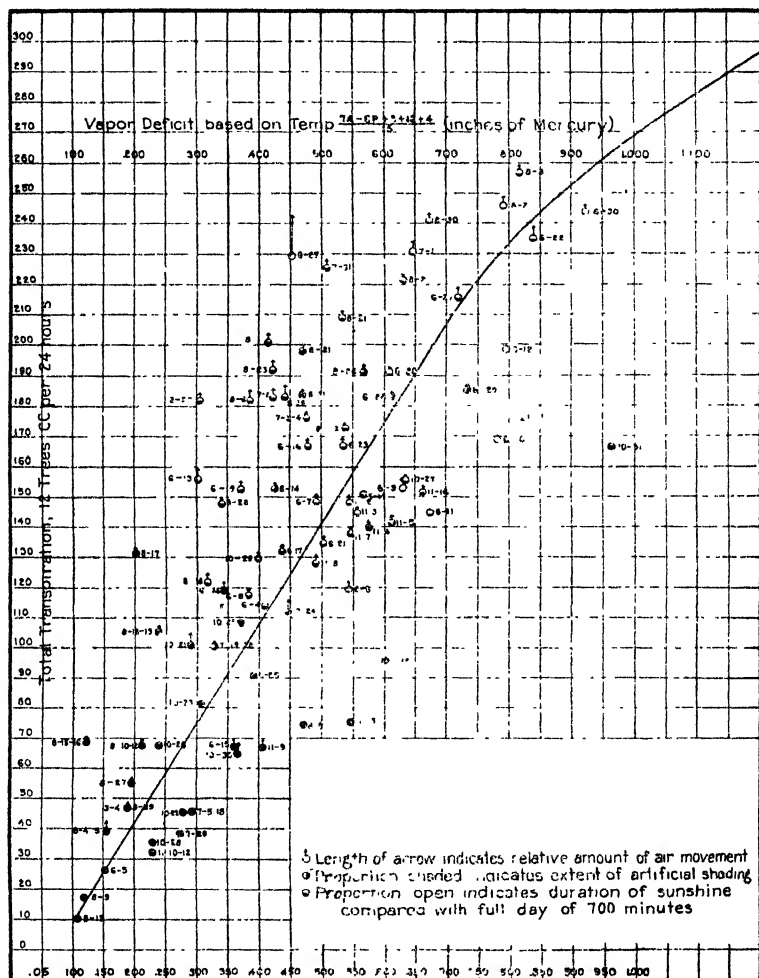


FIG. 1.—Transpiration in relation to saturation deficits in 1927. Numbers opposite circles give month and day of observation.

In figure 1 the results of such computations of vapor deficit for 84 periods during the season of operations are plotted with the daily transpiration sums. The basic data will not be tabulated, since it is voluminous and has no precise value. Instead, the general character of each period is indicated by symbols on the diagram. The mean curve which may be obtained from the 84 points is only suggestive in showing—

1. That for a given saturation deficit a wide variation is possible in the amount of transpiration induced. It is believed, however, that this is largely the result of insufficient data for computing both saturation pressure in the leaf and atmospheric vapor pressure.

2. Air movement increases transpiration somewhat more than has been allowed by the computations, which, in fact, made no allowance for this factor except as it might influence the depression of the psychrometer wet bulb.

3. The amount of sunlight is not the cause of much variation in the results. If anything, the use of black-bulb temperatures has made a little too much allowance for sunlight effects; or, in other words, the plants are not quite so strongly affected by light as is the black-bulb temperature.

4. The transpiration rate (average) is quite proportionate to vapor deficits until the former approaches a daily amount equal to the total weight of the plants, when transpiration, apparently, does not quite keep up with saturation deficits.

5. The transpiration at the end of the season is less than in the earlier months, days of like valuations being compared. The size of the plants, of course, increased during the season, but the increase in leaf area was almost wholly during the first month. It seems safe to say that old leaf tissues do not permit as much transpiration as young tissues.

6. The physical control of transpiration and the lack of plant control is fairly evident, though by no means proved.

#### TRANSPIRATION IN 1920

The striking differences between the species, indicated by the transpiration rates in 1917, both on the basis of growth accretion and relative leaf areas or leaf exposures, called, first, for a reasonable explanation of the temporary circumstances which produced such results, and, secondly, for a repetition to determine whether similar relations of the species might hold with new and different material and whether either the absolute or relative water requirements might be different under more natural environmental conditions.

The first need had been definitely pursued in the interim, and the second was fulfilled during the summer of 1920. In repeating the transpiration tests, it was especially sought to have as large an assortment of plant material as could be adequately dealt with.

#### MATERIAL STUDIED

Twenty-three pots of plants were used, compared with the 12 in 1917. Of these one containing a large yellow pine failed early in the season. An additional pot without trees served to measure the possible direct water loss from the soil. In a number of cases, two or three specimens were handled as one in a single pot, in order to give a better average result. (See Plates 4 to 6.)

The limber and bristlecone pines and a single large spruce specimen were from the same lots of stock which were drawn upon in 1917, having been in the Fremont Station nursery during the interval. The remaining trees were all younger stock grown in the same nursery, and mainly of fairly definite origin as to seed. Where possible a variety of seed sources was represented in the selections for each species, in order to determine the possibilities of variation between geographic forms of the same species.

The data on the trees used are given in Table VI.

TABLE VI.—Source, size, growth, and condition of trees in 1920 transpiration test

Species and source of seed.	Age.	Num-ber pot-tered.	Green weight	Similar specimens dried	Cal-cu-lated dry weight of pot-tered trees.	Weight at end of season				Seasonal weight gain.		Leaf ex-posed from pho-to-graph.	Remarks.	
						Num-ber	Dry-green ratio	Green.		Total dry	Green.			Dry.
								Living plant.	Air-dry sal-sage.					
	Years.		Gm		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Sq cm.		
Yellow pine:														
Harney, S. Dak	4	2	21.46	2	.394	8.46	21.29	0.95	22.24	9.45	0.99	86	22	Slow in beginning vernalion.
Do.	4	2	18.84	2	.394	7.43	22.19	.37	22.56	8.88	3.72	85	1	Do.
Bitterroot, Mont	4	2	15.34	2	.400	6.14	20.64	.22	20.86	7.86	5.52	107	18	
Tusayan, Ariz	4	2	17.78	2	.377	6.70	27.40	.42	27.82	9.66	10.04	114	15	
Douglas fir:														
Hayden, Wyo	8	1	28.20	2	.419	11.81	28.44	.84	29.28	13.60	1.08	144	17	Almost no small fibrous roots, but some new growth made.
Leadville, Colo	8	1	34.65	1	.408	14.13	31.86	.22	34.08	15.15	—	140	10	Large proportion of new foliage.
Pike, Colo	8	1	21.30	2	.410	9.08	23.17	.20	23.37	9.86	2.07	142	7	
Lodgepole pine:														
Colorado, Colo	5	2	19.81	2	.400	8.10	28.16	.20	28.36	9.91	8.55	111	3	
Washakie, Wyo	5	2	22.27	2	.405	9.02	32.90	.46	33.36	11.84	11.09	282	23	
Gunnison, Colo.	5	2	32.61	2	.390	12.72	45.69	.32	46.01	16.53	13.40	381	24	
Engelmann spruce.														
San Isabel, Colo	4	3	11.80	7	.392	4.66	20.28	.32	20.60	7.33	8.71	103	9	
Montezuma, Colo	4	2	5.38	6	.391	2.10	10.30	.10	10.40	3.99	5.02	189	19	
San Isabel, Colo	4	3	7.27	2	.388	2.82	13.02	.12	13.14	4.90	5.87	84	12	
Unknown	8	1	55.69	1	.434	24.60	76.15	.77	76.92	30.64	21.23	295	11	Much slower than small spruces in beginning vernalion.
Larmer pine:														
Unknown	9	1	64.18	2	.448	28.76	73.36	.68	74.04	30.93	9.86	217	16	Buds barely extended during season; roots grew reasonably well.
Do.	9	1	32.47	2	.448	14.55	31.13	.28	31.41	14.64	—	119	21	
Bristlecone pine:														
Unknown.	9	1	29.80	1	.444	13.23	31.59	.22	31.81	14.69	2.01	146	8	
Do.	9	1	12.55	1	.444	5.58	15.12	.06	15.18	6.06	2.03	102	4	
Scotch pine:														
Rye variety	4	1	29.70	1	.394	11.70	31.30	.26	31.56	13.60	1.86	191	13	
Do.	4	1	21.40	1	.394	8.44	27.50	.63	28.13	11.21	6.73	277	2	
Siberian larch:														
Russia	6	1	9.87	2	.298	2.94	12.90	.98	13.88	5.87	4.01	98	6	Slow in beginning vernalion.
Do.	6	1	15.38	2	.298	4.38	16.51	1.42	17.93	7.66	2.55	133	20	[Nearly all of foliage fallen and dry when final weight was determined, No. 6 dried earlier than No. 20.
All	...	34	537.84	47	400	...	640.90	12.04	...	...	125.10	47.54	...	...

# SOIL

The soil in which the trees were potted was nearly the same as that used in 1917. There had been added to the original soil a somewhat more clayey sand of granitic origin, so that in 1920 we find a greater water-holding capacity, a slightly lower moisture equivalent, and a third higher wilting coefficient. The data on the 1920 soil are:

	Per cent.
Saturation .....	36.46
Capillarity.....	31.02
Moisture equivalent. .	10.42
Wilting coefficient (average for spruce and yellow pine).....	4.88

It was planned to carry about 12 per cent moisture, and to prevent the saturation of the lowest stratum of the soil by injecting the daily supply of water near the surface. The feed tube, then, instead of opening into the inverted clay pot at the bottom of the can, was bent about 2 inches below the surface and opened into the soil near the center of the can. Aside from this the potting arrangement was the same as in 1917.

The 12 per cent moisture would constitute, in the average of the 22 cases, 284.4 gm. of water. At the outset, however, the average pot was given 528.8 gm. of water, in order to create a very favorable condition. In the average case this supply lasted much longer than had been anticipated and was not brought down to standard until after July 1, so that during the first two months the moisture conditions were by no means uniform.

Finally, beginning September 3, the water content was gradually reduced, until on September 27 it was 70 gm. below the standard, or in the average case amounted to 9.05 per cent. This change was designed to simulate the usual autumn drying of the soil.

We thus have the following average conditions in the several pots, comparing with a mean availability of about 0.850 through the entire season of 1917:

May 11, 528.8 gm. = 22.31 per cent = availability 0.780; decreasing to 284.4 gm., or 12 per cent, about July 1 = availability 0.594; reaching September 27, 214.4 gm., or 9.05 per cent = availability 0.461.

It is, therefore, seen that in the osmotic sense the conditions for ready absorption of the water were far less favorable in 1920 than in 1917.

# PROCEDURE

The procedure in handling the pots was in minor details almost the same as in 1917. On the other hand, as has been mentioned, they were not under glass but under a canvas cover which was raised during the entire day except when showers occurred, permitting unmodified sunlight to reach the trees and also allowing much freer air circulation, the primary effect of which was, undoubtedly, to prevent the occurrence of excessive temperatures. While the extreme sun temperatures recorded were not materially lower than in 1917, the air temperatures were scarcely above those outside the shelter and were very much lower than in 1917, when they averaged 6° F. higher than the outside air.

The revolving table on which the pots were exposed was nearly 5 feet in diameter and made a revolution every two hours.

## RESULTS

## Amount of Transpiration Compared with 1917

The first point to be noted in Table VII is that the amount of transpiration in 1920 was very much less than in 1917.

On the basis of mean green weight it was 42.4 gm. in 1920, as compared with 123.0 in 1917. On the basis of leaf exposure it was 8.81 gm. in 1920, as compared with 20.6 in 1917. For leaf area we have no data in 1920. It is evident, however, that, considering the amount of water used in relation to size of trees, the transpiration was only one-third to two-fifths as great in 1920 as in 1917. This may be accounted for—

1. By a season of only about 147 days for the average tree in 1920, as compared with 203 days in 1917.

2. By reason of much lower air temperatures in 1920.

3. By reason of considerably less sunshine in 1920, but especially the lack in June, when the driest atmosphere usually prevails. The following data for whole months give the sunshine in recorded minutes:

	1917.	1920.
May.....	8,622	9,598
June.....	15,807	8,903
July.....	12,932	11,310
August.....	10,496	11,704
September.....	10,442	11,695
Total.....	58,299	53,210

It is also probable that on account of the arrangement of the room the trees received a smaller percentage of the total sunshine in 1920.

4. The water of the soil was less readily obtainable in 1920.

TABLE VII.—Actual water losses and transpiration in relation to size and growth in 1920

Species	Yellow pine				Douglas fir		
Pot No	22	1	18	15	17	10	7
Transpiration.							
May 11 to 31.....	51.5	52.8	35.6	66.2	83.0	108.9	112.0
June.....	109.0	112.0	102.7	144.6	111.4	122.9	134.0
July.....	132.4	163.1	236.4	280.0	117.6	162.6	149.0
August.....	101.2	236.8	338.2	492.7	130.3	180.0	177.0
September.....	151.4	292.8	313.0	509.4	130.9	190.3	181.8
October (to day indicated)	(7)	(8)	(8)	(7)	(8)	(5)	(6)
	31.6	80.5	73.7	105.9	47.8	32.1	39.7
Total for season ..	637.1	939.0	1,099.6	1,598.8	621.0	796.8	793.5
Correction, direct evaporation	79.4	79.6	79.6	79.4	79.6	77.7	79.0
Net transpiration ...	557.7	859.4	1,020.0	1,519.4	541.4	719.1	714.5
Transpiration.							
Grams per gram weight accretion—							
Green.....	715	231	185	151	501	.....	345
Dry.....	564	592	615	514	302	506	992
Grams per gram mean green weight ..	26.1	41.8	56.8	67.3	19.1	21.6	32.1
Grams per square centimeter leaf exposure ..	6.5	10.1	9.5	14.2	3.8	5.1	5.0

TABLE VII.—*Actual water losses and transpiration in relation to size and growth in 1920—Continued*

Species.	Lodgepole pine.			Engelmann spruce.			
Pot No. ....	3	23	24	9	19	12	11
Transpiration:							
May 11 to 31. ....	64.5	83.7	94.5	125.9	93.5	99.6	181.8
June. ....	154.8	246.6	243.2	177.3	143.3	127.9	327.7
July. ....	196.3	548.3	607.1	212.1	149.4	194.1	607.8
August. ....	394.0	726.0	923.6	341.3	240.5	272.5	894.5
September. ....	435.1	627.5	708.9	363.6	224.8	227.8	926.0
October (to day indicated)	(8) 103.1	(6) 106.8	(6) 118.7	(5) 56.2	(7) 42.3	(5) 29.8	(5) 123.8
Total for season. ....	1,347.8	2,338.9	2,696.0	1,276.4	893.8	951.7	306.6
Correction, direct evaporation	79.6	79.0	79.0	77.7	79.4	77.7	77.7
Net transpiration ..	1,268.2	2,259.9	2,617.0	1,198.7	814.4	874.0	2,983.9
Transpiration							
Grams per gram weight ac-							
cretion—							
Green. ....	148	204	195	138	162	149	140
Dry. ....	701	802	688	450	431	420	494
Grams per gram mean green							
weight. ....	52.9	82.0	60.9	74.6	103.8	86.1	45.3
Grams per square centi-							
meter leaf exposure	11.4	19.0	15.4	11.6	12.0	10.4	10.1

Species.	Limber pine		Bristlecone pine		Scotch pine.		Siberian larch.		All.
Pot No. ....	16	21	8	4	13	2	6	20	
Transpiration									
May 11 to 31. ....	131.2	79.2	57.3	74.3	81.7	39.1	71.9	69.2	...
June. ....	151.2	77.2	105.3	128.1	149.1	121.6	158.1	152.8	...
July. ....	321.3	84.1	128.8	114.5	183.4	231.0	236.2	165.6	...
August. ....	484.4	112.9	132.2	107.5	194.7	416.0	457.0	322.4	...
September. ....	534.4	125.2	108.4	87.6	212.7	454.7	123.5	152.8	...
October (to day indicated)	(8) 112.5	(7) 11.4	(3) 7.1	(3) 6.7	(6) 38.2	(8) 102.1	...	...	...
Total for season. ....	1,755.0	510.0	539.1	518.7	859.8	1,364.5	1,046.7	862.8	...
Correction direct evaporation	79.6	79.4	76.0	76.0	79.0	79.6	70.3	70.3	...
Net transpiration. ....	1,675.4	430.6	463.1	442.7	780.8	1,284.9	976.4	792.5	247,940
Transpiration.									
Grams per gram weight									
accretion—									
Green. ....	170	...	230	168	420	191	243	311	198
Dry. ....	773	4,785	317	434	409	464	333	257	522
Grams per gram mean green									
weight. ....	24.4	13.5	15.1	32.0	25.6	52.6	94.0	49.7	42.4
Grams per square centi-									
meter leaf exposure. ....	7.0	3.6	3.7	6.3	5.0	11.9	10.0	6.0	8.81

On the basis of the growth made, the transpiration is also less in 1920 than in 1917, though not so strikingly so. For green-weight accretion the figures are 198 and 263, and for dry-weight accretion 522 and 880, respectively, for 1920 and 1917. In other words, for green-weight accretion it required 75 per cent as much water in 1920 as in 1917, and for dry-weight accretion 59 per cent as much. The difference between these two percentages and between the two years may be due largely to the fact that very little foliage falling in 1917 was salvaged and accounted for, while in 1920 this was carefully done. However, it is believed the amount dropped by the trees in 1917 was relatively very small and insufficient materially to affect the results.

It seems fairly evident that the transpiration per unit of growth is a more stable quantity than that per unit of leaf exposure or whole mass, in spite of the fact that the former is very much dependent on the whole leaf area functioning.

## Water Requirements

Comparing now the species, as was done for the data of 1917, we have them in 1920 aligned as in Table VIII.

TABLE VIII.—Comparison of different species as to water requirement

Species	Transpiration per unit of dry-weight accretion	Probable error in average
	Gm.	Gm.
Limber pine.....	2,779	850
Lodgepole pine.....	730	18
Douglas fir.....	600	160
Yellow pine.....	571	5
Engelmann spruce.....	441	6
Scotch pine.....	436	23
Bristlecone pine.....	376	49
Siberian larch.....	295	32

## Resistance to Transpiration

On the basis of leaf exposures we have a very different arrangement (Table IX).

TABLE IX.—Comparison of species as to resistance to transpiration

Species	Transpiration per square centimeter leaf exposure	Probable error in average.
	Gm.	Gm.
Lodgepole pine.....	15.27	0.49
Engelmann spruce.....	11.12	.21
Yellow pine.....	10.08	.66
Scotch pine.....	8.45	2.91
Siberian larch.....	8.00	1.69
Limber pine.....	5.30	1.44
Bristlecone pine.....	5.00	1.10
Douglas fir.....	4.63	.39

## EXPLANATION OF RESULTS

On thorough consideration of the meaning of the results which have been given above for both 1917 and 1920 tests, we come to the conclusion that neither method of comparing the species is very satisfactory when the number of individuals involved is insufficient to cover all possible variations. In these tests considerable variation in growth rate is to be expected as the result of more or less incomplete recovery from transplanting. The small spruces, for example, in 1920 showed no delay in starting new growth; the single large spruce came on satisfactorily after considerable delay; one large limber pine grew vigorously while the smaller one did not extend its terminal or branch buds over one-fourth

inch and put on no new foliage. None of the Douglas firs grew vigorously in 1920 while all of them dropped a good deal of their old foliage.

The water requirements and the rate of transpiration per unit of mass or leaf exposure are closely interrelated, it will be seen, for the following obvious reasons:

1. New shoots undoubtedly transpire more freely than old foliage.
2. When a plant is not growing it seems to transpire relatively little, either because it can not obtain the water or, possibly, because it has closed its stomata.
3. It therefore follows that the amount of transpiration per unit of mass or leaf exposure may be very much affected by the amount of growth made.
4. And it is equally apparent that the transpiration per unit of growth may be somewhat dependent on the total amount of foliage functioning, though it must be conceded that so long as the old foliage transpires, it probably is also capable of some photosynthesis, and therefore contributes to growth.

The important point is to recognize that an extreme case of poor growth may throw the specimen very high in one list and very low in the other list (for example, Pot 21 in 1920). It seems, therefore, only reasonable to eliminate from both records the individuals which have apparently not performed normally in the matter of growth. As the basis for normalcy is so meager, we can not bring ourselves to the elimination of any trees except one limber pine in 1917 and another in 1920.

On the other hand, what is true of individual trees affects the relations of the species. Apparently, small spruces are capable of a generally larger accretion percentage than similar trees of our other native species. As has been pointed out, this would be a very important factor in competition. Its bearing on absolute water requirements and drought resistance is not so plain, and we have had serious misgivings as to the desirability of comparing the species, in their moisture relations, on this basis. Nevertheless, it is fairly apparent that a high growth percentage in itself denotes something of superiority in the relation of the tree to its environment. It indicates either that the tree has some peculiar ability to make use of the available light or that it is more capable than others of supplying the water, or the carbon dioxid, in just the right amount to make photosynthesis effective. If either of the latter is a factor in the result, we may say either that the plant has superior ability to obtain water or that it has superior ability to retain it while keeping the stomata open for the ingress of carbon dioxid. There is left, therefore, little doubt in our minds that the tree of low "water requirement" as related to growth is in fact the tree which has the superior control over its water supply.

It is, therefore, important to compare the species on the basis of the growth made, in order to understand the marked differences between the absolute transpiration rates in 1917 and 1920, which leave the relations of the species so confused.

On comparing further Tables III and VIII, it is seen that with one or two exceptions, namely, yellow pine and bristlecone pine, the water requirements as determined in 1917 and 1920 are not so divergent that we need hesitate to combine them to obtain more effective averages, and it seems best to use the value for each tree in obtaining the mean. There are also given the mean growth percentages for each species. In figure 2 the general relation between growth rates and water requirements is plainly shown—a relation that seems logically unavoidable.



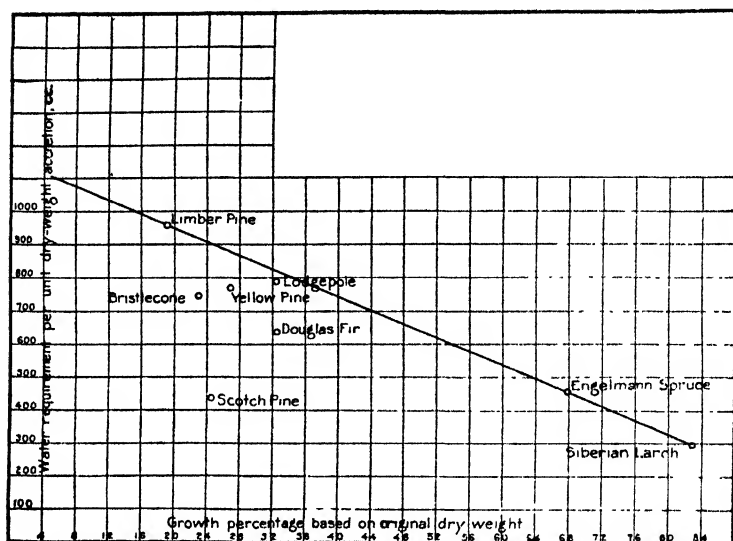


FIG 2.—General relationship between water requirements and growth rates for all species in 1917 and 1920

TABLE X.—Water requirements and growth in 1917 and 1920

Species.	1917			1920			Average water requirement	Average growth percentage <sup>a</sup>
	Number of trees	Water requirement	Growth percentage	Number of trees	Water requirement	Growth percentage		
		Gm.			Gm		Gm	
Limber pine . . .	1	1,144	31.0	1	773	7.5	958	19.2
Lodgepole pine . . . . .	2	954	46.6	6	730	27.8	786	32.5
Yellow pine . . .	2	1,555	32.4	8	571	25.6	768	27.0
Bristlecone pine . . . . .	2	1,110	31.3	2	376	14.7	743	23.0
Douglas fir . . . . .	2	684	64.9	3	600	11.0	634	32.6
Engelmann spruce . . .	2	525	75.2	9	441	66.4	456	68.0
Scotch pine . . . . .	..	..	..	2	436	24.6	436	24.6
Siberian larch . . . . .	..	..	..	2	295	83.5	295	83.5

<sup>a</sup> Dry accretion related to dry weight at beginning of season

At least two important points are gained by the combination of the 1920 with the 1917 data. The position of limber pine, as the least effective user of water, is more nearly established, and lodgepole pine is brought into this class, where our empiric estimates would place it, as we shall see later.

But the main reason for presenting the growth data in Table X is to explain the positions of the species as given in Table IX. It is seen that Engelmann spruce maintained in 1920 almost as high a growth rate as in 1917, and this explains its appearance as a relatively extravagant user of water on the leaf-exposure basis. Conversely, limber pine and Douglas fir both appear as conservative users of water in 1920, evidently because they were relatively inactive.

In combining, then, the data for the two years of transpiration per unit of leaf exposure, we shall not lose sight of the fact that the positions of Douglas fir and limber pine, at least, and to a lesser extent that of bristlecone pine, are determined by relative inactivity in 1920, and that they really belong higher in the scale than here shown. There are possible means of making allowance for this influence of growth activity on the total transpiration, but they are so purely arbitrary that we hesitate to use them.

TABLE XI.—Mean resistance to transpiration, 1917 and 1920 (growth data repeated to explain relative positions)

Species	Mean transpiration per square centimeter leaf exposure	Mean growth percentages.
	Gm	
Lodgepole pine .. . . .	17.35	32.5
Yellow pine .. . . .	14.96	27.0
Limber pine. . . . .	14.15	19.2
Bristlecone pine .. . . .	13.10	23.0
Engelmann spruce .. . . .	11.97	68.0
Douglas fir. .... .	9.50	32.6

That these relations of the species are not fixed and might easily be modified by additional data or consideration of different climatic varieties is fairly apparent from the divergence of the individual results. On the same basis as Table XI, the three lots of yellow pine studied in 1920, each of which maintained a healthy condition and performed, relatively, much as it had been performing in the nursery, gave the following results:

Variety	Transpiration per square centimeter leaf exposure	Growth percentage
	Gm.	
Harney, S. Dak. . . . .	8.3	11.7
Bitterroot, Mont. .... .	9.5	19.5
Tusayan, Ariz. . . . .	14.2	27.0

There is every reason to believe these results were normal and express something of varietal difference. It is seen that the high growth rate of the Arizona form was accomplished with the extravagant use of water. Whether this is generally true of the more southern forms our data are inadequate to determine. Rather similar differences occur with lodgepole pine, but here the Wyoming form is the most extravagant and rapid growing.

## ABILITY OF THE SPECIES TO OBTAIN WATER

A very considerable amount of light is thrown on the meaning of Table XI and our other discussions by considering the water requirements of each species in 1920, with the considerably less abundant water supply, as a measure of the ability of each to extract water from the soil. Thus, in comparing Tables III and VIII it was particularly noted that bristlecone and yellow pines, which in 1917 were more generous users of water, in 1920 took very low positions in the scale. The relative changes are indicated in the following table, where the use in 1920 is expressed as a percentage of the use in 1917. Only the better growing limber pines are considered. Comparison is made on the basis of Tables III and VIII and Tables IV and IX.

Species	Relative transpiration in 1920	Species	Relative transpiration in 1920.
	<i>Per cent</i>		<i>Per cent</i>
Engelmann spruce. ....	77.2	Limber pine. ....	48.5
Lodgepole pine. ....	66.2	Yellow pine. ....	33.0
Douglas fir. ....	57.6	Bristlecone pine. ....	28.8

In some degree these observations are corroborated by the data in Table XII in which is shown the change in relative transpiration rates through a period in which the last pot listed for each species was given additional water. For the periods represented by September 28, 29, and 30, the water in each pot was at 70 gm. below standard, and except for the one pot of each species, the same amount was maintained on succeeding days. On the morning of September 30 the amounts in the special pots were increased 50 gm., and on October 1 they were brought up to standard. After this, they were not again watered until dried out to the original basis. If, then, the availability of the water, which was thus increased from about 0.461 to 0.594, as described under the heading "soil," has a bearing on the amount transpired, its effect should in all cases be apparent in the transpiration recorded October 1 and 2. In the table is shown what was the prevailing relative amount for the special tree, compared with others of the same species for the three days when water contents were the same. The relative amount on October 2 is then shown as a percentage increase.

It is to be noted that after October 2 the relative rates of the specially watered trees steadily declined, and in some cases went below the previous rates, until the day indicated by "W," when the first fresh water would take effect. This is plainly due to exhaustion of water close to the roots and is a commentary on the importance of transport within the soil.

TABLE XII.—Effect on transpiration of increasing the available water <sup>a</sup>

Species	Pot No.	Transpiration measured on the morning of—								Average relation Sept. 28 to 30.	Per-centage increase Oct. 2.
		Sept. 28.	Sept. 29.	Sept. 30.	Oct. 1.	Oct. 2.	Oct. 3.	Oct. 4.	Oct. 5.		
Yellow pine.....	1	11.0	10.8	10.3	10.5	12.9	12.0	13.4	9.5	11.6	34.4
	18	10.6	9.8	12.0	10.8	11.1	10.8	13.1	9.7	10.1	
	22	5.4	4.5	5.1	4.9	5.9	5.5	5.6	4.5	5.0W	
	15	18.7	17.4	19.5	21.2	28.1	20.3	19.4	10.5	13.6	
Relative amount in No. 15 <sup>b</sup> .....											
		.693	.694	.712	.810	.940	.718	.605	.443	.510	0.700
Douglas fir.....	7	6.3	6.4	6.3	7.0	8.0	8.7	8.6	6.5	7.9	{ 44.8 30.5
	10	6.8	5.7	7.5	6.2	8.0	8.6	8.9	6.6	.....	
	17	4.3	3.7	5.1	6.4	7.0	8.0	7.4	5.8	.....	
Relative amount in No. 17 <sup>b</sup> .....											
		.328	.306	.370	.485	.437	.462	.423	.443	.....	.335
Lodgepole pine.....	3	15.6	14.2	16.4	13.7	17.2	14.7	19.3	12.2	14.0	33.9
	23	21.9	22.9	23.5	20.1	24.6	21.1	24.5	17.7W	18.9	
	24	23.1	19.9	23.8	24.4	32.6	27.3	23.4	16.5	18.9	
Relative amount in No. 24 <sup>b</sup> .....											
		.616	.537	.597	.722	.780	.763	.535	.552	.593	.583
Engelmann spruce.....	9	12.1	12.6	13.8	12.3	14.7	13.3	15.3	12.9	.....	8.8
	12	7.2	6.9	7.3	6.4	8.1	6.5	8.3	6.9	.....	
	19	6.9	6.8	7.6	7.2	8.1	6.8	8.7	6.5	.....	
Relative amount in No. 11 <sup>b</sup> .....											
	11	32.4	30.5	35.5	34.5	40.7	32.9	27.9	22.3	.....	1.211
	.....	1.237	1.160	1.237	1.332	1.318	1.237	1.158	1.180	.....	
Limb pine.....	21	4.5	3.6	4.2	4.5	6.0	5.3	5.5	4.0W	5.5	14.5
	16	16.9	16.4	17.8	20.2	28.8	24.0	21.3	13.1	16.3	
	.....	3.76	4.56	4.24	4.49	4.80	4.53	3.88	3.28	2.96	
Relative amount in No. 16 <sup>b</sup> .....											
											4.19

<sup>a</sup> Last pot for each species increased 50 gm. effective October 1 and 20 gm. more effective on morning of October 2.

<sup>b</sup> The amount in the pot receiving extra water is expressed as a ratio to the total in other pots of same species.

From the data in Table XII we see that the limber pine was little stimulated, probably because its transpiration is always moderate. The spruce was still less affected, apparently because it is always able to satisfy its needs. Lodgepole, Douglas fir, and yellow pine were about equally helped and seemed greatly invigorated. The relatively high transpiration of the fir on October 1 and 3 can be accounted for only by an error in weighing.

To a certain extent, these performances may be accounted for by the root habits of the trees. It is possible that the finely divided and numerous roots of the spruce give it immediate control over so much more soil that it exhausts the available water much less quickly than those species which usually develop only a few coarse roots. To some extent this would also explain the ability of limber pine to obtain its water more steadily. On the other hand, either lodgepole or Douglas fir ordinarily has much better roots than yellow pine, yet these three were about equally stimulated by a heavy addition to the water supply.

On the whole, this matter is only suggestive and does not, we believe, explain the relative behavior of the species. That Engelmann spruce possesses a remarkably great ability to supply itself with all the water that is needed under the most trying circumstances, and that this ability is exceeded, among the species studied, only, possibly, by that of Siberian larch, seems proved beyond a shadow of doubt. This is plainly shown in the day-to-day records where, if there is a marked contrast in the amount of sunlight on two succeeding days, or in other conditions conducive to high transpiration, it is the spruce which is invariably able to live up to these conditions most fully. Thus we are enabled to say quite confidently that the relatively high rate of transpiration of spruce on cloudy days, as shown by Table V, truly expresses an ability to make use of all available light and does not signify that this species is unable to meet the conditions which cause high transpiration from all the species.

#### SUMMARY

It has seemed desirable to go into this matter fully on account of the complicating features introduced by the radically different results secured in 1917 and 1920, and in order that we might not deceive ourselves as to the true meaning of the results. It has been necessary for us to go through with this analysis in order to reach a conclusion, and it is hopeless to expect the reader to reach a conclusion by any other process.

It now becomes fairly apparent that transpiration is very much dependent on water supply and that the relatively low water use of some of the species in 1920, when the water supply was maintained at a low level, is not to be considered as a virtue but rather as evidence of a lack of ability to supply needs. And, even though in some cases growth may not have been seriously impaired by the inability of certain species to keep the leaves well stocked with water, yet it is perfectly evident that the species which show this inability in the most marked degree would soonest succumb in time of real drought or in the usual autumnal drought that occurs where there is strong competition.

There are, apparently, two slightly different problems to be considered in comparing the species. The one has to do with the relative requirements of different tree species of a unit size. The other has to do with

the amount of water required during the production of a unit of growth. Both relations are important in ecology. But, except with the weed trees, limber and bristlecone pines, we have found no essential difference in comparing the species on the two bases. To what extent a low water requirement means great drought resistance we shall see later. Apparently there is not a great deal of difference between the species at the minimum water point. There is evidently a great difference in their activity or vigor under better conditions, and this, perhaps, is the most important point we have brought out.

The important consideration is that the additional data secured in 1920 have not materially altered our conception of the physiological qualities of the species, which are best indicated relatively by Table III. It is true that in 1920 lodgepole pine used relatively more water and made less vigorous growth, so that now, by either Table X or XI, it appears as a more extravagant demander than yellow pine. Likewise Engelmann spruce kept up its rate in 1920 more nearly than Douglas fir and hence appears more extravagant. These facts, however, merely confirm the belief that the species which under favorable moisture conditions is most conservative is best able under all conditions to satisfy its needs. The reason for this will be more apparent after considering sap density and its osmotic bearings.

To summarize, briefly, for the species, what it is believed is shown by the preceding data and discussions:

1. Limber pine: Very slow growing, but also very conservative in the use of water. Represents highest development in structural protection against atmospheric conditions, but probably poor development in relation to the soil. Not adapted for competition.
2. Bristlecone pine: Not quite so far developed as limber pine in any respect mentioned.
3. Yellow pine: Relatively slow grower and has little protection against losses; consequently from either standpoint its water use is very high. Shows little ability to cope with drought conditions. Arizona form more vigorous and equally extravagant of water.
4. Lodgepole pine: More rapid grower than yellow pine.
5. Douglas fir: Apparently adapted to conserve water but growth rate not nearly equal to spruce, possibly being in these tests more adversely affected by transplanting because of the relatively long roots, which are characteristic, and their small numbers.
6. Engelmann spruce: Most highly developed of our native species to make use of all conditions of environment in vigorous growth. Is conservative of water and low in water requirements for growth. These characteristics may partly explain its shade tolerance and its success in competition.
7. Siberian larch: Although little studied, seems to be developed even beyond spruce in all particulars.
8. Scotch pine: Stands about midway between our pines in transpiration rate and lower than any of them when growth is considered. Seems to be developed along lines of spruce and fir for alpine conditions. It should be remembered we are speaking only of the Riga form.

#### SAP DENSITY AND THE VARIATION IN TRANSPIRATION RATES

Sometime before the transpiration tests which have just been described were made in 1917, carefully conducted drying tests on green and partly

dried lodgepole cones, in a calorimetric kiln, had clearly demonstrated that the amount of heat required to extract a gram of water from cones was not 536 calories but an amount always in excess of that, which increased rapidly as the amount of sap in the cones decreased through preliminary drying. This apparent increase in the latent heat of vaporization, it was thought after a study of the physical chemistry of solutions, might be related to the phenomena of rising boiling points and decreased vapor pressure with increases in the concentration. Unfortunately, no direct experimental work on this problem had been done, so far as I have been able to learn to date, and a number of physicists consulted agreed that in their interpretation of the theory of solutions a solute could not increase the latent heat of vaporization of the solvent.

The writer, in the most dependable tests it has been possible to make, has found that at the respective boiling points of various concentrations of common salt in water, the latent heat of vaporization decreased slightly with increased concentration, up to the point of saturation. Making allowance for the higher boiling point of the concentrated solution, it would appear that for a given temperature the latent heat of vaporization was practically a constant. In these tests an electric immersion heater was employed for the heat supply, the wattage being precisely measured; the evaporation was directly measured by weighing the solution; and allowance was made for direct radiation from the solution and vessel.

The greatest objection to these tests, or to any that we have so far been able to devise, lies in the difficulty of maintaining a constant temperature with a constant and measurable heat supply, at a relatively low temperature such as plant tissues may experience, and also at a relatively low temperature such that the radiation factor is not a great possible source of error. Until these difficulties are overcome we can hardly say that the problem of the latent heat of vaporization as it relates to plant evaporation has been satisfactorily treated.

On the other hand, it is a fairly simple matter, at either high or low temperatures, to determine that the rate of evaporation is very materially reduced with increase in concentration when the source of heat is outside the solution. This would make it appear that there may be a problem in conductivity quite as important as, if not more important than, that relating to latent heats. The resistance to drying, shown by rather concentrated solutions such as sirup, is quite well known. As an illustration of what we mean by heating from the outside, let us take the case of two identical evaporating dishes placed over a steam bath. The steam is constantly in contact with the bottoms of the dishes. It may not, however, give up its heat unless the surface of the dish is being cooled by radiation above or evaporation of the liquid in the dish. Such an exposure can not possibly give any indication of the quantity of heat utilized in evaporating from the dish.

Under such circumstances as these the writer found the evaporation from a saturated salt solution to be less than one-twentieth as rapid as that from pure water.

Similarly, exposing a number of test tubes mainly to the heat of the air in contact with them, the rates of evaporation were found to be depressed by somewhat dilute saline and sugar solutions.

Vessels and bottles in which the contents have been heated primarily by the rays of the sun have not shown any consistent depression of the evaporation rates due to solutes.

The available facts, then, which have a bearing on the possible influence of sap concentration on the rates of evaporation from similar bodies are:

1. At any given temperature the vapor pressure over a solution decreases as the concentration increases, indicating that the solution does possess a stronger hold on the molecules than does the pure solvent and that therefore the solute may at least decrease the rate of evaporation. There is nothing in the quantities involved, however, to indicate that this might be an important factor within the limits of cell-sap concentrations.

2. Calorimetric tests on the heat required for drying cones indicate an increase in the latent heat of vaporization as the concentration of solutions in the cone cells increases. Even admitting that the large number of tests puts the facts practically beyond question, there may be here a case of adsorbed water rather than a case of solutions, and with the molecular affinities and possible latent heats of the former we are not, just at present, concerned.

3. Carefully conducted tests on free saline solutions indicate that the latent heat of vaporization is not appreciably affected by concentration or at least not more so than might be deduced from paragraph 1.

4. Observations on the heating and evaporation of solutions by low-temperature, exterior sources indicate an inability, increasing with concentration, to absorb and transmit such heat in a manner conducive to evaporation. We shall not attempt to go into the theory of this. The important fact is that the heat of the air, and sunlight so far as it is absorbed by the exterior walls of the leaf or the interior cell walls, may be relatively ineffective in producing evaporation from a concentrated as compared with a dilute solution, while, apparently, such rays as were directly absorbed within the solution would be about equally effective in all cases. It goes almost without saying that, if such absorbed heat does not produce evaporation, it must increase the temperature of the leaf until a point is reached where absorption and radiation balance.

In view of these facts, when, at the close of the transpiration tests, it was discovered that the several species showed such unaccountable and surprising differences in transpiration rate, with respect to growth or mass or leaf area, the first thought was that they must exhibit differences which could be expressed in the qualities of the solutions from which the evaporation of water takes place.<sup>5</sup> This thought was too hastily transformed into action by igniting the specimens which had served for the transpiration tests, in the expectation that the ash weights would be an index to the solutes in the plants and the densities of their cell solutions. This supposition was, of course, erroneous in taking no account of the soluble carbohydrates as well as some of the mineral oxids which would be lost in ignition and which comprise the greater mass of the solutes. As indicated in Table I, the ash percentages are irregularly variable and are found to bear no relation to transpiration rates.

Having destroyed the best source of information on the physical qualities of the original specimens, the next step was to obtain specimens as nearly as possible like those used in the transpiration tests. This was done by securing trees of the same classes as those taken from the nursery in the spring of 1917 which had spent the growing season in the nursery.

<sup>5</sup> The writer wishes to acknowledge the very helpful suggestions of the article by Barrington Moore (19), which was received in galley proof at such a time as to aid very materially in solving the current problem, and which reviews a number of the more recent researches on this problem.



The specimens were collected on December 3, 1917, before the ground was frozen, and when there had as yet been no drying winds. From three to eight trees of each species were taken so as to secure a considerable mass of material. The tops were cut off at the root collar and those parts only were used.

#### PROCEDURE IN DETERMINING SAP DENSITY

The necessary data for determining the momentary density of the sap in a plant appeared to be—

(1) The weight of the green material, determined as quickly as possible after the material was collected.

(2) The weight of the soluble matter leached out with an abundance of water and evaporated.

(3) The weight of the insoluble pulp, oven-dried.

By adding together (2) and (3) and deducting from (1), the original amount of water (and other solvents) in the plant is obtained, and this, when divided into (2), gives the sap density, usually expressed as a percentage.

In these original tests the plant material for each species was ground to a pulp, and these pulps were allowed to stand in cold water of about 10 times the pulp volume, 3 waters being used for each. Finally the pulp was all accumulated on a filter and dried with the filter paper. The aqueous solutions were evaporated at temperatures not exceeding that of boiling water. In all cases the so-called "sugars" thus secured, after becoming dry, were not wholly resolvable, indicating that colloidal matter had been included and had passed through the filters. This matter was a small proportion of the total solids, however, and may be assumed to have equally affected all samples.

#### RESULTS

The sap density percentages obtained for the nursery seedlings, in the first tests made, were as shown in Table XIII, in which the water requirements are again given. Figure 3 shows that the sap densities and water requirements plot a curve which is remarkably perfect, considering the changes that have been noted in relative transpiration rates during the season, and the somewhat questionable value of the water requirement for limber pine, which must be based on the performance of only one specimen.

TABLE XIII.—*Sap densities and water requirements in 1917*

Species.	Sap density in material of Dec. 1, 1917 (tops only).	Water re- quirements of trees in transpira- tion tests.
	Per cent.	Gm.
Limber pine.....	19.6	1,144
Yellow pine.....	21.8	1,555
Bristlecone pine.....	22.4	1,110
Lodgepole pine.....	23.2	954
Douglas fir.....	27.9	684
Engelmann spruce.....	29.5	585

At first thought it does not appear probable that the density of the spruce sap, which is only one-half greater than that of yellow pine, is sufficient to reduce the relative water loss of spruce to one-third or one-fourth of that of the pine. It is not necessary to assume that the small water loss of spruce, as related to its photosynthetic activity and amount of growth, is entirely the direct result of the physical properties of the dense sap of this species. To determine how the result may be brought about, all the following factors must be given consideration:

1. Higher sap density means less evaporation per unit of available heat.
2. Higher sap density means higher leaf temperatures before evaporation can take place at a given rate, with the possibility that in sunlight the leaf may become warmer than the air and therefore lose heat by radiation and conduction.

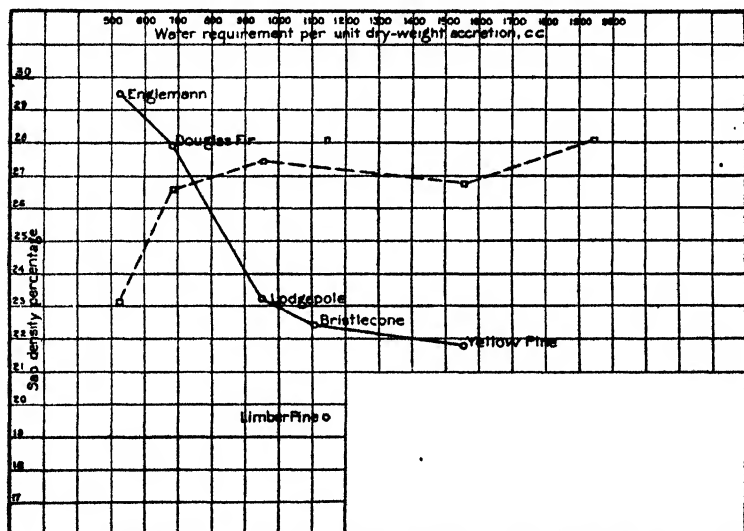


FIG. 3.—Water requirements in 1917 transpiration tests compared with sap densities on nursery specimens, December 3, 1917 (solid line) and sap densities in natural habitats, December 30, 1917 (broken line).

3. Higher sap density, by permitting higher leaf temperatures, should facilitate the photosynthetic process, thus relatively reducing the water requirement.

4. It must be equally true that greater photosynthetic activity or capacity will tend toward higher sap density as well as increased growth.

When, therefore, the question is asked, "Is the high sap density of spruce a direct cause of its low transpiration rate, or is the former merely a concomitant of greater photosynthetic activity, and is this last the really important physiological characteristic?" we are compelled to reply that the three things are so interdependent that all are equally causes and effects. It is left almost beyond question, however, that of the species we have considered the spruce represents the highest development and that this development is expressed in the highest growth rate (first columns of Table X) in the greatest current accumulation of soluble carbohydrates at the time of the December examination and in the most

effective use of water throughout the season. On the other hand, when we consider the low sap density of limber pine and its low water requirement in relation to either mass or leaf area, we obtain a suggestion that transpiration may be controlled by mechanical means rather than through the physical properties of the sap and that such control indicates a low state of development because it inevitably means the sacrifice of the absolute growth rate. Yellow and lodgepole pines, with relatively low sap densities, appear not to exert the mechanical control over transpiration and are, as a result, perhaps more fastidious as to growing conditions than limber and bristlecone pines.

While it seems important to have demonstrated that among the species of approximately equal development from the forester's standpoint, growth, photosynthetic activity, sap density, and the relative extravagance in water use are thus interrelated through simple physical control, yet the really important question is whether high or low sap density exerts a control over the more absolute water loss. In considering this it seems unquestionably best to use the leaf-exposure basis, since the maximum area exposed to the sun must determine very largely the total amount of energy which might be available for the evaporating process. Without repeating the data which are given in Tables II and XIII (omitting the slow-growing specimen of limber pine), the relationship is shown in figure 4. It is to be noted that the transpiration rate of yellow pine on this basis is relatively high, while bristlecone and limber pines are relatively low. In 1920, these relations are completely reversed. The facts leave little doubt that high sap density does materially suppress transpiration.

#### RESULTS IN 1920

In view of the apparent relation between transpiration rate and internal condition of the tree, it is important to see whether the physical characters which we might ascribe to the several species are in any degree constant. Let us first consider the transpiration material of 1920.

To obtain better data on the sap density of the trees whose transpiration was observed during 1920, sample trees corresponding to those potted were treated at the beginning of the season, and the transpiration trees were themselves treated at the close of the primary test.

With the freshly dug trees at the beginning of 1920 it was possible to grind and treat the whole plants in very much the same way as the tops were treated in December, 1917. Owing to the large number of lots involved, however, four sets of sample trees were merely dried, and it is necessary to deduce their approximate sap densities from other results for the same species.

In the fall examination it was considered of first importance to determine the dry weight of the trees, as a measure of growth, without the risk of losing any material or whole results through accidents. The trees were, therefore, first oven-dried. It was several months before opportunity presented itself to bring out this dried material, grind it in a mortar, make the extractions of sugars, and again dry the leached pulp. In this case no attempt was made to evaporate and weigh the sugars. This method is probably open to the criticism that a longer period is required to secure the same degree of leaching of solutes that may be expected with green material, and also that the drying of the material probably coagulates and holds some colloidal matter that would, from green material, pass off with the solutes. This may account in part for the relatively low sap densities found at the end of 1920.

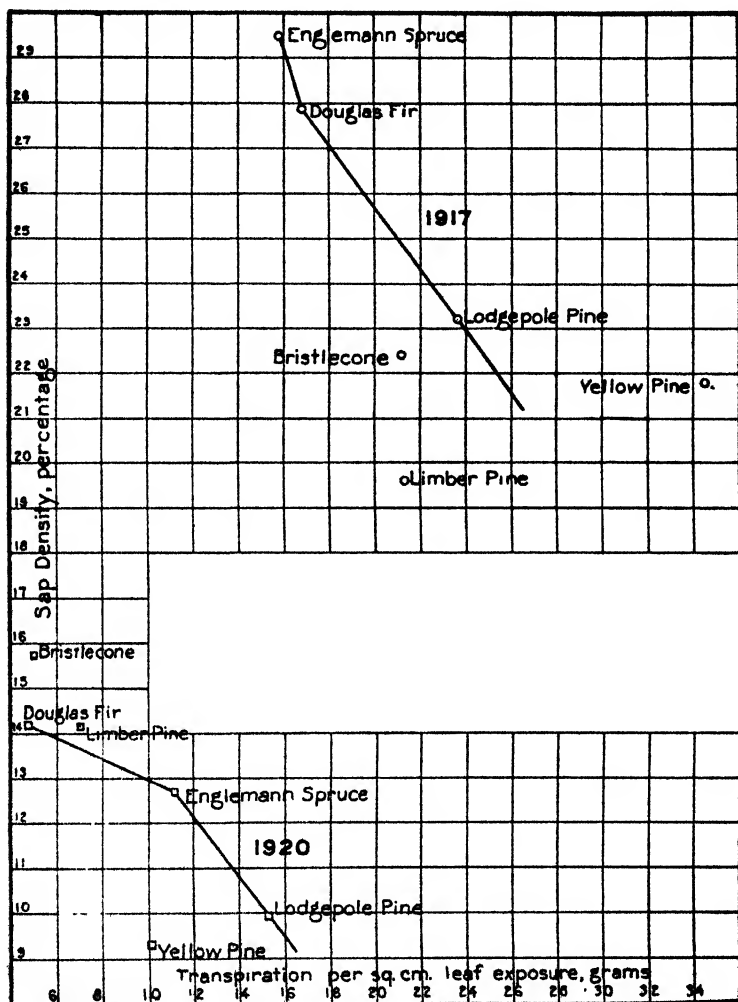


FIG. 4.—Relation between sap densities and transpiration rates on basis of leaf exposures.

The results of the two determinations are shown in Table XIV.

TABLE XIV.—*Sap density of trees in 1920 transpiration tests (whole plants)*

Species.	Pot No.	Source.	Sap density at—		Seasonal change.
			Beginning of season	End of season	
Yellow pine . . . . .	22	Harney . . . . .	<i>Per cent.</i> 13. 15	<i>Per cent.</i> 16. 18	
	1	do . . . . .	<i>a</i> 13. 15	15. 59	
	18	Bitterroot . . . . .	<i>b</i> 13. 50	13. 01	
	15	Tusayan . . . . .	12. 71	9. 30	
Average . . . . .			13. 13	13. 52	+0. 39
Douglas fir . . . . .	17	Hayden . . . . .	15. 24	15. 63	
	10	Leadville . . . . .	14. 78	13. 25	
	7	Pike . . . . .	<i>b</i> 15. 50	13. 71	
Average . . . . .			15. 17	14. 20	- . 97
Lodgepole pine . . . . .	3	Colorado . . . . .	14. 72	10. 60	
	23	Washakie . . . . .	<i>b</i> 14. 65	8. 80	
	24	Gunnison . . . . .	14. 49	10. 38	
Average . . . . .			14. 55	9. 93	-4. 62
Engelmann spruce . . . . .	9	San Isabel . . . . .	13. 70	Lost.	
	19	Montezuma . . . . .	<i>b</i> 13. 65	12. 84	
	12	San Isabel . . . . .	9. 91	12. 93	
	11	Unknown . . . . .	13. 50	12. 32	
Average . . . . .			12. 69	12. 70	+ . 01
Limber pine . . . . .	16	Unknown . . . . .	16. 05	13. 02	
	21	do . . . . .	<i>a</i> 16. 05	<i>c</i> 15. 36	-1. 86
Bristlecone pine . . . . .	8	do . . . . .	15. 75	16. 29	
	4	do . . . . .	<i>a</i> 15. 75	15. 68	+ . 23
Scotch pine . . . . .	2	Russia . . . . .	10. 43	10. 80	
	13	do . . . . .	<i>a</i> 10. 43	12. 65	+1. 29
Siberian larch . . . . .	6	do . . . . .	8. 54	13. 75	
	20	do . . . . .	<i>a</i> 8. 54	<i>d</i> 15. 05	+5. 86

*a* One test only for this class of material

*b* Sugar not extracted. Sap density estimated from green-dry ratio.

*c* The specimen of high sap density is the one that made practically no growth

*d* These sap densities determined after foliage was nearly all air-dry. Making allowance for this the sap densities should be about 2 per cent less.

In considering Table XIV it should be borne in mind that at the time of potting the trees for transpiration study some of the species had made very appreciable growth while others had probably felt the stimulus of spring very little. It is perfectly apparent from other data secured that the initiation of growth in the spring means a flooding of the plant with water. Thus the relatively low sap densities of spruce and Siberian larch are expressive of their response to relatively low temperatures, while that of yellow pine is more indicative of a low condition throughout the winter.

In view of the fact that the stage of the seasonal advance had affected the several species differently, it is questionable whether the spring data, or the changes throughout the season, have any great value in our present comparisons.

On the other hand, if the sap densities in the fall may be taken as indicative of conditions existing during most of the season, certain striking facts are in evidence. It has been mentioned that the abundant moisture supplied the trees in the spring, and the relatively dry condition later on, may have induced the production of a great deal of new tissue which the trees were not, later on, able to supply with adequate building material. This would seem most markedly the case with all the lodgepoles and with the Tusayan yellow pine whose growth was so vigorous. It is believed, therefore, that the evidence is fairly convincing that in 1920 either the moisture was not sufficiently available to permit effective photosynthesis in some of the species or that the sunlight and temperatures were below par in effectiveness. Possibly it is a combination of these things which left sap densities very low at the end of the season. Still, in comparing the absolute values with those of 1917, it should be remembered the earlier values refer only to the tree tops.

These sap densities are found to bear a broad relationship to the water requirements of the several species, though this is not so well defined as in 1917, probably because of the seasonal changes in water supply and the less favorable light conditions. Comparing the mean post-season sap densities with the transpiration per unit of leaf exposure, however, and again omitting the questionable limber pine, we have the data in Table XV, which have already been illustrated in figure 4.

TABLE XV.—*Transpiration and sap densities in 1920*

Species	Transpiration per square centimeter leaf exposure	Mean post- season sap density
	Gm.	Per cent.
Lodgepole pine.....	15.27	9.93
Engelmann spruce.....	11.12	12.70
Yellow pine.....	10.08	9.30
Scotch pine.....	8.45	11.72
Siberian larch.....	8.00	14.40
Limber pine.....	7.00	14.19
Bristlecone pine.....	5.00	15.78
Douglas fir.....	4.63	14.20

It is again evident in the 1920 results that the effectiveness of sunlight in producing evaporation from leaves must be very considerably affected by the density of the sap involved. If we were to balance the variations in one season against those in the other, it is readily seen that the relationship would be almost perfect. To what extent these variations may be due to error in determining either sap density or leaf exposure must remain a question until a great deal more material has been examined. It does seem certain, however, that the relative positions of the species, in regard to either sap density or transpiration rate, are by no means constant. The best that can be done at present is to accept average values for each species, as has been already done in considering the transpiration independently. The explanations, already made, of variations in growth in the two seasons should be considered in connection with the variations in sap density.

## STABILITY OF SAP DENSITIES

It has already been indicated that the sap density of a given species is by no means constant. In order that progress may be made in the use of the sap density measure, or the osmotic pressure determination, as an indicator of the relations between plant and environment, it is extremely important to realize (1) that the tree may pass annually through a definite cycle of changes and (2) that the current sap density may be quite largely influenced by current atmospheric conditions as well as water supply. If the tree were always able to supply as much water as was demanded by the losses at its leaves, then only a cyclic change would be apparent, dependent on cell division and photosynthesis, or primarily on temperature and light conditions. It might be said, therefore, that the species which shows the least fluctuation with current atmospheric conditions is the one best able to supply any demand for water, and it would seem that this species would best endure a long drought in the soil.

As to the cyclic change in sap density, it would seem that the following describe the general conditions of the seasons which bring it about:

1. In the spring we have rising temperatures and increasing duration and intensity of sunlight, which alone would increase the osmotic pressure in favor of the plant as against the soil. Coupled with this there is usually an abundant supply of moisture in the soil immediately after its thawing, often augmented by the melting of large masses of snow and by heavy rains. These conditions cause a heavy inrush of water and, because the atmospheric demands are at this season very moderate, an extreme turgescence of the tissues already formed becomes not only possible but unavoidable. It is believed this turgescence is the foundation for cell expansion and division, from which the new tissues arise.

2. With the advance of spring and advent of summer the moisture supply of the soil is usually much reduced, but even if this were not the case the formation of new tissues might be checked because of the very great increase in transpiration, due both to greater atmospheric demands and water losses from the new succulent tissues already formed. In the conifers, outward evidences of growth usually disappear abruptly early in the summer. The period of warmest weather, then, is not one for the formation of new tissues, but for the creation of the materials with which those already formed may be built up and solidified. Fruiting, of course, also demands some of these materials. The sap density should, therefore, increase from the moment that cell division becomes negligible.

3. The autumn season in temperate climates is almost universally the least favorable as regards current precipitation, and at this season the soil water is most likely to have been completely exhausted. For this reason the amount that can be supplied is often inadequate for all the transpiring members, and we witness the drying and falling of all deciduous leaves and of the oldest ones on the evergreens. Unless the water supply in the leaves becomes so low as to prevent the chemical processes, photosynthesis in the evergreens is not checked, and sap densities may be expected to reach their highest points, partly because the actual amount of water present is less than at any other season.

4. While under some circumstances the winter season may be one of almost complete dormancy, with photosynthesis stopped by low temper-

atures and little light and the movement of water stopped by freezing of the soil, such a condition does not describe the winters of the region in which we are particularly interested in this study. Here the winter days are often warm and bright enough to permit possibly some photosynthesis in the evergreens and certainly very considerable transpiration. Such days may be followed by severe cold of sufficient duration to freeze the tree and the soil to a depth of 1 or 2 feet. These cold waves are not uncommonly followed by warm winds which immediately thaw the foliage and may make great demands on its moisture before the tree stems and the soil thaw out enough to provide a new supply. Thus, in extreme cases great injury may be done, and in the usual weather cycles the tree is subjected to very marked changes in water supply and in the sap density of its foliage. At this season the sap density, it will be readily seen, may depend very greatly on the exposure of the tree, since the primary factor in drying is wind. A tree growing in a dense stand with a northern exposure passes through much more moderate changes than one in an open stand where both sunshine and wind may have full effect.

#### Sap Density in Period of Vernalion

We have already pointed out, in connection with Table XIV, the possible bearing of different responses to temperature on the comparative sap densities in the spring of 1920. In Table XV are given additional data which, with the explanatory notes, make a very clear case. It is not surprising to find that a control test made with nursery material collected June 1, 1918, when growth of most species had begun, shows complete disarrangement of the December, 1917, values.

The material was collected as before, complete aerial portions being taken. These were placed immediately in stoppered bottles and were exposed each to the others' vapor until June 18, in order that from the losses or absorptions some idea as to relative osmotic pressures might be gained. The exposure was not long enough to give more than an indication. After this period the material was dried in the bottles, then leached and redried. Spruce specimens were inadvertently omitted from this collection.

TABLE XVI.—*Sap densities of nursery specimens at beginning of growing season, 1918 (tops only)*

Species.	Age	Number of tests.	Average sap density	Relative osmotic pressure indicated by vapor transfers.
	Years		Per cent.	Per cent.
Limber pine.....	7	2	15.54±1.37	0.978
Yellow pine.....	2-6	2	15.10±0.55	.998
Bristlecone pine <sup>a</sup> .....	7	1	20.37	.980
Lodgepole pine.....	4	2	12.22±0.53	.979
Douglas fir.....	6	2	16.91±0.41	.969
Siberian larch <sup>b</sup> .....	4	2	10.61±0.98	.972
Western white pine <sup>a</sup> .....	3	1	20.33	1.014
Scotch pine.....	3	1	13.48	.984
Aspen (new leaves).....	0	1	14.18	1.012

<sup>a</sup> Buds not yet opened.

<sup>b</sup> One specimen with and the other without roots. The former showed the lower density.



The data indicate that the beginning of new growth had permitted the essential equalizing of sap densities, except with bristlecone pine and western white pine. It is difficult to see why the density for bristlecone pine should have remained high without giving it, apparently, a high osmotic pressure. The very thin sap of Siberian larch is accounted for by the advancement of its growth, which always begins earlier than that of any of the native species.

TABLE XVII.—*Sap density of trees in natural habitats after drying weather, December, 30, 31, 1918*

Species.	Site where collected.	Sap density.
		<i>Per cent.</i>
Limber pine .....	2 southwest slope .....	28.67
	6 northwest slope .....	29.86
	6 northwest slope .....	26.42
	12 ridge .....	27.08
	9 north slope .....	28.39
	All .....	28.08 ± 0.45
Yellow pine .....	2 southwest slope .....	<sup>a</sup> 25.64
	2 southwest slope .....	<sup>a</sup> 29.52
	6 northwest slope .....	27.46
	4 east slope .....	25.30
	12 ridge .....	25.84
	All .....	26.75 ± 0.59
Lodgepole pine .....	1 south slope .....	27.24
	8 ridge .....	27.61
	All .....	27.42 ± 0.16
Douglas fir .....	2 southwest slope .....	27.35
	4 east slope .....	26.40
	12 ridge .....	28.10
	9 north slope .....	24.44
	All .....	26.57 ± 0.56
Engelmann spruce .....	3 northeast slope .....	24.38
	5 bottom .....	22.78
	5 bottom .....	22.16
	All .....	23.11 ± 0.51

<sup>a</sup> On the morning of Feb. 23, 1918, corresponding specimens showed 27.05 per cent and 25.12 per cent, respectively, for these two trees. The specimen of lower sap density in each case was from a young, vigorous tree; that of higher density from a low limb of an old tree badly infested with mistletoe.

On the whole, while the determinations of osmotic pressure are not complete, it is indicated that they were essentially the same when the sap densities were nearly the same. We may, therefore, feel safe in assuming that for other conditions relative osmotic pressures will be about proportionate to sap densities, which might not be the case if the solutes of different species were materially different in composition and molecular weights. A boiling-point test made with accumulated solutes from all the species shows that an osmotic pressure of about 19 atmospheres may be expected when the sap density is 20 per cent. Freezing-point deter-

minations have also shown that with all of our conifers a sap density of 1 per cent is approximately equivalent to 1 atmosphere of osmotic pressure, this relation holding at least up to 20 per cent. Such tests have not, as yet, been sufficient to bring out any consistent differences in the saps of different species.

#### Winter Sap Densities in Natural Habitats

Attention may now be turned to determinations of the sap density of trees growing in their natural habitats, as made at the end of December, 1917. The foliage specimens were collected on the afternoons of December 30 and 31, both days being warm and the soil not yet frozen in any instance at a depth of a foot. The preceding week had been warm and dry, with a high evaporation rate for that season. Hence it may be expected that the results will show the influences of different exposures.

All material was from limbs at a height of about  $4\frac{1}{2}$  feet above the ground.

In this, as in all the following cases where only foliage is sampled, the outer half or two-thirds of the needles was clipped off with shears in sections about one-half inch long. This material was leached before drying and was otherwise treated as the ground pulps had been.

When these results are compared with those obtained from nursery stock on December 3 (fig. 3), it is seen that a very great but regular difference in the value exists. The average sap density of limber pine has increased 8 per cent, of yellow pine 5 per cent, of lodgepole 4 per cent; that of Douglas fir has decreased 1 per cent and that of spruce 6 per cent. These changes form almost a straight line when plotted with the original sap densities as abscissae.

This shows that sap densities in a given species are subject to great variations, but it does not mean that they have no significance. The differences between these field specimens and the nursery trees growing under uniform conditions reflect the fact that the pines had lately been subjected to the strongest drying influences, while the fir in part, and spruce wholly, had recently been protected from any severe drying. Also, owing to the protection afforded the latter species at all times, they had probably never had the benefit of full light and, therefore, may not have accumulated as large a supply of carbohydrates as the pines growing in the open.

To assume from this that spruce or fir is not subjected in the field to drying stresses equal to those experienced by the pines, or that the former would not tolerate great stresses as well as the latter, is altogether erroneous. These tests were made before the soil was frozen and before the winter exposure had had opportunity to bring about any degree of equilibrium between different sites. It is greatly to be regretted that this series of specimens could not have been duplicated late in the winter.

#### Winter Sap Densities Near Timber Line

On the other hand, specimens collected at high elevations, on January 1, 1918, only a day or two later, tell a very different story, for here the soil was already deeply frozen, and the exposure to evaporation had been very severe for the preceding six days.

These results and others which show changes with season, weather, and soil conditions are given in Table XVIII.

TABLE XVIII.—*Sap densities in exposed situations, at high elevations, 1918*

Species	Location	Sap densities.			
		Jan. 1.	Feb. 6.	May 7.	May 18
Engelmann spruce	Station F-16 . . . . .	<i>Per cent.</i> <sup>a</sup> 37.30	<i>Per cent.</i> 31.29	<i>Per cent.</i> <sup>b</sup> 33.60	<i>Per cent.</i> 22.16
Bristlecone pine	do . . . . .	30.75	28.68	<sup>c</sup> 19.71	20.84
Limber pine	Station F-13 . . . . .	31.00	24.00	.....	.....
Bristlecone pine	Cabin Creek . . . . .	30.56	30.37	<sup>d</sup> 21.70	21.50
Engelmann spruce	Gulch near F-13 . . . . .	.....	23.66	.....	19.02
Alpine fir	do . . . . .	.....	21.45	.....	20.62
Limber pine	Cabin Creek . . . . .	.....	.....	.....	21.07
Engelmann spruce	Cabin Creek, edge of water.	.....	.....	.....	24.51

<sup>a</sup> Similar specimen obtained Jan. 15, 1921, from a very exposed site showed 33.5 atmospheres of osmotic pressure by freezing-point method with 27.7 per cent sap density.

<sup>b</sup> Twigs from which needles were clipped showed at same time 19.95 per cent.

<sup>c</sup> Twigs from which needles were clipped showed at same time 15.84 per cent.

<sup>d</sup> Twigs from which needles were clipped showed at same time 15.19 per cent.

At Station F-16 there is at all times a contrast between the spruce and bristlecone pine specimens, until May 18, when thawing had become general. This is probably not altogether a specific difference but is due in part to the fact that the spruce was located in a hollow which collected snow and did not permit even temporary thawing of the soil until very late, while the bristlecone was on high ground only a few feet distant, from which the snow is usually swept away, and which might, therefore, thaw in a brief period.

It may be a very significant fact that although the soil temperatures at Station F-16 on February 6 were 21.5° F. at 1 foot, and 23.6° at 4 feet (as against 27.0° and 30.5° on January 1), and although there had probably been no thawing whatever during January, the sap densities of both spruce and bristlecone pine decreased during the month. The evaporation rate from February 1 to 6 was only about one-fifth as great as from December 26 to January 1, meaning, of course, much less current desiccation in the later period. But it is also indicated that at some time between January 1 and February 6 the leaves must have obtained moisture from some source. This might have been by transfer from the stems, if thawing of aerial parts occurred; but there is no apparent reason why the leaves should not have taken up vapor from the atmosphere during periods when the vapor of the atmosphere was practically saturated. The possibilities of such absorption, as a relief for winter drought conditions, are too important to be overlooked.

The material collected May 7 showed in all cases much lower sap density in the twigs than in the more exposed half of the needles, the latter being selected because logically subject to the greatest drying. This indicates that small variations in the results may be expected from clipping slightly more or less than half of the needles.

All specimens showed a decided drop in densities on May 7, by which time there was a great deal of surface thawing, except, as explained, around the roots of the spruce at Station F-16. This tree responded in the next period.

The spruce and alpine fir obtained from a protected stand in a gulch near F-13 both on February 6 and May 18 show plainly the advantages

of protection. The high density of the last spruce in the test, on May 18, is difficult to explain, in view of the exposure on a southwesterly bank and the apparent abundance of moisture. This may be due, however, both to the full exposure of the tree to light and to a possible high degree of nonavailability of the moisture as a result of acidity and lack of aeration.

Summarizing, it is evident that the sap density of any species or individual is not a stable quality but varies according to the amount of photosynthesis which is permitted and according to current conditions affecting water supply and transpiration. Nevertheless, there is found in these data no reason for changing the original conclusion that, given equal opportunities and exposures, the sap density of spruce will be higher than that of any of the other species; that spruce will tolerate a very great loss of moisture, and a resultant high sap density in the needles, without injury; and that it can, therefore, be said that spruce is not only better equipped to resist transpiration, other things being equal, but that the ability to resist transpiration and its possibly injurious effects makes spruce in reality the least moisture-demanding of all of the Central Rocky Mountain trees.

These conclusions, however tentative, must at least develop a wariness to accept average or temporary moisture conditions of the site as *prima facie* evidence of the relative moisture requirements of the species occupying it.

On the other hand, we have as yet no evidence that spruce is more drought-resistant than other species or that the sap density and the specific qualities that affect it react upon distribution through the water requirements. All that we have so far been able to show is that low sap density permits a species to occupy warm sites where the exposure is very great most of the time, while high sap density appears to hold the species to cool sites, where the winter drought may be severe, especially at high altitudes.

#### WILTING COEFFICIENTS FOR DIFFERENT SOILS AND SPECIES

If it could be shown that one species is capable of extracting the moisture of the soil to a lower point than other species before wilting or other injury to the plant was apparent, this would constitute direct evidence that the first species not only was less likely to experience fatal drought conditions but also was capable of sustaining higher internal osmotic pressures without injury to the protoplasm. When wilting occurs, if the condition has been approached gradually, it may be assumed that the osmotic pressure in the plant is essentially the same as in the soil, and the latter, of course, increases as the moisture content decreases and the concentration of the soil solution increases.

It has already been indicated in connection with transpiration in 1920 that spruce appeared to be able, under all conditions, to obtain the water required for free transpiration more nearly than any of the pines or Douglas fir. A similar test with the moisture gradually reduced to complete nonavailability would, perhaps, be preferable to wilting tests, which must be conducted with seedlings in order that the end-point may be observed ocularly. It is fairly evident that the seedlings may not show development of the internal characters which are important in this connection to the same extent as older trees. However, it can not be gainsaid that it is the seedlings which are subjected to the greatest dan-

gers, and it is their performance, rather than that of older trees, which determines the composition of forest types.

Although only a few wilting tests have been made in which the several species have been observed growing in the same soil, a considerable amount of information has been obtained on each species in a variety of soils; and by reference to the physical properties of these soils we may obtain fairly satisfactory comparisons. In each case, the species used was that one which occupied the given soil or predominated in the type in the field.

#### PROCEDURE

In general the intention has been to secure the wilting coefficient for the soil as found in the field—that is, with the normal admixture of rocks and gravel, since the saturation and capillary capacities and other physical measurements were on this basis. In this respect mountain soils present difficulties ordinarily not met with in agricultural work.

To attain this end it is not sufficient to sample the soil for moisture content after the seedlings have wilted. The moisture content must be determined for a mass of soil large enough to represent normal proportions of rock and finer material.

Pans about 10 inches square were used in the earliest tests, the soil being in a layer from 1 to 1½ inches deep. These were sometimes found to be too shallow to accommodate the rocks which should be included; consequently a standard round pan was specially made, having a diameter of 7 inches, a depth of 3 inches, a soil depth of 2½ inches, and an ordinary soil weight of about 4 pounds. A few holes were punched in the bottom of each pan to prevent excessive wetness and to aid aeration, it being the belief that with the pans on a bench the evaporation rate through these small holes could never be an important factor. In fact, though the soil surfaces have usually been paraffined, it has never been attempted to make the coatings air-tight, since the object is not to prevent water loss from the soils but to insure that when wilting occurs the moisture distribution throughout the soil shall be fairly uniform.

The vegetation has been secured by sowing seeds of the desired species in the pan of soil, watering these moderately, and permitting the seedlings to develop for about a month before coating the pans and cutting off the moisture supply. Beyond the age of a month the seedlings may rapidly lignify, so that the wilting does not occur promptly or is very difficult to detect. This is true of Douglas fir seedlings at any age. It is, of course, realized that seedlings of this age may not exercise the same control over moisture as would older trees.

The soil sample is placed in the pan in an air-dry condition and is oven-dried to determine its net weight. This practice may have had some effect on the colloids but is fully justified by the assurance it gives that micro-organisms will be eliminated and will not cause the untimely death of the seedlings. However, as mountain soils are rarely strong in clay, as the samples have always been air-dried first, and as the oven temperature has been only 92° C., it is thought any change in soil qualities may virtually be ignored.

After drying, a cupful of soil is taken from the pan, a weighed lot of seeds is strewn over the smooth surface of the remaining soil, and the cupful is then used to cover the seeds.

The moisture applied to induce germination and development of the seedlings has usually been left wholly to judgment, the intention being to give all that can be used and never to permit the surface to become dry.

The seedlings have been developed with abundant sunlight, in the greenhouse, avoiding excessive temperatures as far as possible. To make a satisfactory test each pan should develop at least 100 seedlings.

When the final weight of a pan is secured, with the seedlings wilted, deductions are made for the known weight of paraffin applied, as also for the weight of the seed used, which is assumed to be the same as that of the wilted seedlings and the loose hulls. This weight could generally be ignored without affecting the result appreciably, for the moisture content is usually 60 or 80 gm., as against 1 to 5 gm. for the seed of any species except yellow pine.

#### DIRECT COMPARISONS OF THE SPECIES

Not until 1920 was it possible to conduct special tests with two or more species in the same soil. The most comprehensive test, and therefore the least likely to be misleading, was conducted from April to September, 1920. In this case the soils were not sterilized by oven-drying, and considerable damping off of the seedlings occurred, which may be confused with legitimate wilting in the early stages. The pans were watered and weighed daily, and the losses of seedlings were recorded, so that it is possible to consider the losses at any stage. Because of the damping off, and also to make these results more comparable with those in which but one wilting period was recorded—that is, the time when practically the entire number collapsed—it seems best to consider in all these more recent tests the mean wilting point for the last 25 per cent of the total number of seedlings observed. Not infrequently the weakest seedlings die with twice as much water available as is required to sustain the strongest.

In this particular test the moisture equivalents of the five soils were determined first, under a force of 100 gravity; and, assuming that these quantities were indicative of the same degree of availability in each soil, the watering in each case was so gauged as to maintain this moisture equivalent. It may be remarked that this quantity was very favorable for germination and establishment. Later the water content of each pan was reduced to two-thirds of the moisture equivalent, finally to one-third, and from that point downward by 5 gm. stages. This is important because other tests indicate that the drought which a seedling will tolerate depends much on the moisture to which it has become accustomed.

The soils in this case were not paraffined, but some water was given almost every day in order to eliminate, so far as possible, extreme drying-out of the surface. It is significant that, perhaps on this account, the wilting coefficients are relatively lower than usual.

The results of this test, which have already been given in the research manual (4, *pl. 1*) to illustrate the relation between wilting coefficient and moisture equivalent, are given in slightly different form in Table XIX.

We shall not discuss the rather variable relations of these wilting coefficients to the physical measures of moisture-holding properties of the soils. Suffice it to say that other evidence points to the fact that the several wilting coefficients represent an osmotic constant in the different soils, while either the capillary moisture or moisture equivalents fall considerably short of this. This relationship will be discussed in connection with the field-moisture problem. The physical measures of soil moisture bear only a general relation to wilting coefficients and must be used with this understanding.<sup>6</sup>

<sup>6</sup> The reader is urged to note the discussion of this by Bates and Zon (4) where it is made plain that a constant ratio between wilting coefficient and moisture equivalent is impossible if a wide variety of soil types is considered.

The important thing shown by Table XIX is that the wilting coefficients of yellow pine, Douglas fir, and Engelmann spruce are essentially the same in all the soils, while that for lodgepole pine is much higher. There is, moreover, no evident reason for the fact that in some of the soils (sandstone and prairie shale) the wilting coefficient for spruce is lower than for either pine or Douglas fir. We must, at least at this stage, regard these variations as accidental.<sup>7</sup>

It will now be well worth while to determine whether, as between any two of these apparently equal species, a greater number of results brings out any difference. A very considerable amount of data has been secured on Douglas fir and spruce growing in the same soils. In introducing these data it is desirable to point out:

1. That ocular observations on wilting, especially when the moisture supply is steadily declining, tend to favor Douglas fir, because that species has a much stronger and more fibrous stem and rarely collapses. The evidence of wilting is, therefore, much less plain than in the frail spruce seedling, and, it seems likely, may not be obtained until a day or two after the fatal condition has first existed.

TABLE XIX.—*Wilting coefficients of the four important species in 5 types of soil*

Kind of soil	Capillary moisture	Moisture equivalent 100 grav-ity	Mean wilting coefficients, best 25 per cent of the seedlings.				
			Yellow pine	Lodgepole pine.	Douglas fir	Engelmann spruce	Average of four.
Granitic gravel sandy loam	Per cent 26.58	Per cent 10.55	Per cent 2.28	Per cent 2.42	Per cent 2.16	Per cent 2.26	Per cent 2.28
Composite limestone loam	31.85	22.00	3.23	4.12	3.56	3.50	3.60
Composite sandstone loam	35.34	21.77	4.10	5.29	4.30	3.93	4.40
Prairie silt loam from shale	37.77	28.79	7.80	8.69	7.79	7.43	7.93
Composite lava silt loam	43.16	27.80	4.52	6.00	4.97	4.87	5.09
Average			4.39	5.30	4.56	4.40	4.66

2. That in the early stages of development Douglas fir roots more strongly than spruce and its roots reach a greater soil area, but particularly in these pan tests they reach the deepest layer of soil which may not be drawn on at all by the spruce if the wilting is accomplished at an early age. (See Plate 7, B.)

3. Therefore, in these tests it is evident that if spruce seedlings tolerate as great a degree of drought as fir, the moisture being known only through the whole pan weight, it must be through greater ability to extract water from the soil.

From Table XX it will be evident that the wilting coefficient for spruce is, on the whole, higher than that for Douglas fir. The difference is only about 3 per cent of the value for fir. Of the 23 cases cited, only 6 give spruce a lower value than fir, and 5 of these 6 are among the loose gravels

<sup>7</sup> Since the foregoing statement was written very convincing results have become available showing the different effects of each of these soils in stimulating the growth of each species, both according to chemical composition of the soil and the concentration of its solution. It can hardly be questioned that this has a direct bearing on the behavior of the seedlings as the wilting point is approached, and in fact that this entire problem is quite as much one of chemical relations as of the physical relations which have been discussed in this paper. It is hoped that something may be published on this chemical phase in the near future.

or sands of granitic origin, while only 1 is found in the more loamy soils. This is at least suggestive that in the soils of freer capillary movement the very meager root system of spruce is not so great a disadvantage. From these facts we certainly can not draw the conclusion that in the osmotic sense spruce has any less control over soil moisture than fir.

INDIRECT COMPARISONS OF THE SPECIES

A very considerable amount of information has been secured in the somewhat routine process of determining the wilting coefficients for a large number of soils of almost every possible origin in connection with nearly every study in which soil quality or soil moisture has been an important factor. For the most part the wilting coefficients have been determined for each soil only with respect to one species, that one being the species which characterized the soil or site in the field. It is obviously necessary, before these results may be used for a comparison of the species, that each result should be related to some other measure of the moisture-holding properties of the soils, and the best measure at present available for any considerable number of the soils is the moisture equivalent at 100 gravity. As we have seen in Table XX, however, even this does not bear a constant relation to wilting coefficients, when radically different types of soil are considered. Particularly does it seem that the coarse-grained granitic soils of the Pikes Peak region, which we have studied more than any other, have an unusually weak hold on the water until the amount is brought close to the wilting coefficient, so that the moisture equivalents of these soils are relatively low.

TABLE XX.—Comparative wilting coefficients of spruce and Douglas fir in the same soils

Kind of soil	Moisture equivalent	Wilting coefficient		Ratio of wilting coefficient to moisture equivalent	
		Fir	Spruce	Fir	Spruce
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>		
Granitic gravels over 50 per cent rocks and coarse gravel, less than 20 per cent silt and clay.	3.53	1.30	1.39	0.368	0.394
	4.35	1.73	1.94	.398	.446
	4.86	2.05	2.03	.422	.418
	5.03	1.77	1.80	.352	.358
	5.04	2.14	2.23	.424	.442
	5.06	2.54	2.34	.502	.462
	5.19	2.01	1.87	.387	.360
	5.57	1.94	1.71	.348	.307
	5.62	2.13	2.61	.379	.464
	11.05	2.94	2.91	.266	.263
Granitic sandy loam . . . . .	11.68	2.72	2.88	.233	.246
Granitic spruce soils, varying from rocky coarse sand to silt loam.	11.72	2.53	2.79	.216	.238
	14.45	3.42	4.09	.239	.283
	19.95	0.41	6.44	.321	.323
	20.32	5.60	5.78	.276	.284
	22.02	5.25	5.61	.238	.255
	26.30	8.34	8.17	.317	.311
	29.84	7.44	7.98	.249	.268
	42.72	17.09	17.40	.400	.407
	73.50	17.53	18.56	.238	.253
	11.96	5.13	5.20	.429	.435
Quartz latite rocky sandy loams. .	13.98	6.96	7.14	.498	.510
	14.85	5.72	6.04	.385	.407
Average of all. . . . .				.3427	.3537
Mean difference. . . . .					.0110
Probable error in mean difference. . . . .					.0029



TABLE XXI.—Miscellaneous wilting coefficients.

## YELLOW PINE

Sample No.	Station or forest.	Origin and character of soil.	Moisture equivalent.	Wilting coefficient.	Ratio of wilting coefficient to moisture equivalent.	Conditions of test.
			<i>Per cent.</i>	<i>Per cent</i>		
326	Nebraska .....	Aeolian ridge sand .....	3.72	0.54	0.145	Without paraffin. Do.
325	do .....	Aeolian bottom very fine sand ..	12.36	2.51	.203	
70	Fremont F-2 .....	Granite gravel .....	5.41	1.16	.214	
71	do .....	do .....	5.81	2.63	.445	
72	do .....	do .....	5.86	2.67	.450	
73	do .....	Granite sand .....	5.29	3.67	.395	
78	Fremont F-4 .....	Granite gravel .....	5.20	1.79	.344	
79	do .....	do .....	4.60	1.28	.273	
80	do .....	do .....	3.70	1.43	.387	
26	Fremont F-6 .....	do .....	6.73	2.44	.363	
29	do .....	do .....	5.24	2.65	.506	Do. Do. Do. Do. Do. Do. Do. Do. Do. Do.
25	do .....	do .....	4.48	1.85	.413	
121	Fremont F-12 .....	do .....	5.87	1.99	.339	
132	do .....	do .....	4.62	1.97	.427	
130	Pike M-1 .....	Granite coarse sand .....	8.91	2.36	.265	
124	do .....	do .....	6.61	1.53	.232	
117	do .....	do .....	6.76	1.68	.249	
53	Black Hills .....	Limestone silt loam .....	30.15	15.33	.502	
15	do .....	Sandstone loam .....	16.02	2.58	.161	
62	do .....	Sandstone silt loam .....	22.30	6.41	.287	
63	do .....	Schist loam .....	19.06	6.26	.328	
122	Colorado .....	Sandstone fine sandy loam .....	16.96	9.97	.587	Do. Do. Do. Do. Do. Do. Do. Do. Do. Do.
127	Wagon wheel Gap .....	Quartz latite loam .....	21.95	3.77	.172	
101	Cache .....	Volcanic ash, etc .....	28.23	12.7	.445	
102	do .....	Silt loam .....	28.05	9.89	.342	
103	do .....	do .....	29.66	10.14	.342	
104	do .....	do .....	27.27	10.40	.382	
632	Wagon wheel Gap .....	Quartz latite sandy loam .....	18.43	7.50	.407	
	Fremont .....	Granitic sandy loam .....	10.42	4.09	.392	

## LODGEPOLE PINE

1	Arapaho .....	Granitic loam .....	17.03	7.34	0.431	Without paraffin. Do. Do. Do. Do. Do. Do. Do. Do. Do.
2	do .....	Granitic sandy loam .....	11.32	3.02	.267	
3	do .....	Granitic fine sandy loam .....	16.75	3.50	.209	
4	do .....	do .....	7.63	2.75	.360	
5	do .....	Granitic coarse sand .....	7.75	5.70	.736	
6	do .....	Transported fine sandy loam .....	13.65	2.96	.217	
262	Medicine Bow .....	Gneiss coarse sand .....	13.45	4.72	.351	
263	do .....	do .....	16.25	3.05	.224	
264	do .....	do .....	11.62	2.99	.257	
265	do .....	do .....	7.62	7.6	.100	
533	do .....	Gneiss fine sandy loam .....	12.90	4.69	.364	Do. Do. Do. Do. Do. Do. Do. Do. Do. Do.
540	do .....	do .....	13.78	5.22	.379	
541	do .....	do .....	14.75	4.75	.322	
558	do .....	do .....	13.76	5.61	.408	
559	do .....	Gneiss fine sand .....	10.22	4.00	.399	
127	Wagon wheel Gap .....	Quartz latite loam .....	21.95	4.41	.201	
71	Leadville .....	Sandstone silt loam .....	21.34	1.90	.089	
12	do .....	Sandstone fine sandy loam .....	12.35	2.52	.204	
57	Fremont F-10 .....	Granitic sandy loam .....	9.27	3.55	.383	
48	Colorado .....	do .....	10.39	3.14	.322	

## DOUGLAS FIR

8	Arapaho .....	Igneous fine sandy loam .....	13.31	4.12	0.309	Without paraffin
66	Fremont F-1 .....	Granite gravel .....	4.29	2.31	.538	
67	do .....	do .....	3.60	.93	.258	
68	do .....	do .....	4.34	.76	.175	
69	do .....	Granite sand .....	8.25	2.13	.258	
17	Fremont F-7 .....	Granite gravel .....	4.71	1.26	.268	
19	do .....	do .....	4.94	1.24	.251	
18	Fremont F-9 .....	do .....	5.03	.96	.191	
127	Wagon Wheel Gap .....	Quartz latite loam .....	21.95	5.11	.233	
533	Medicine Bow .....	Gneiss fine sandy loam .....	12.90	4.04	.313	Do. Do. Do. Do. Do.
540	do .....	do .....	13.78	4.88	.354	
541	do .....	do .....	14.75	4.44	.301	
558	do .....	do .....	13.76	5.59	.400	
559	do .....	Gneiss fine sand .....	10.22	3.76	.368	

TABLE XXI.—Miscellaneous wilting coefficients—Continued

## ENGELMANN SPRUCE

Sam- ple No.	Station or forest.	Origin and character of soil	Moisture equiva- lent	Wilting coeff- icient	Ratio of wilting coeff- icient to moisture equiva- lent	Conditions of test.
74	Fremont F-3.	Granitic gravel. . .	7.87	1.54	0.196	Without paraffin.
75	do . . . . .	do . . . . .	6.53	1.51	.231	Do.
76	do . . . . .	do . . . . .	3.99	1.50	.364	Do.
76	do . . . . .	do . . . . .	3.99	1.40	.311	With paraffin
35	Fremont F-5.	do . . . . .	6.74	2.12	.315	Without paraffin
33	do . . . . .	do . . . . .	7.62	2.64	.347	Do.
34	do . . . . .	do . . . . .	6.68	1.98	.297	Do.
110	Wagon Wheel Gap D.	Quartz latite sandy loam .	20.50	7.56	.369	
111	do . . . . .	do . . . . .	17.38	3.59	.207	
112	do . . . . .	do . . . . .	17.72	4.14	.234	
632	Wagon Wheel Gap A-1	do . . . . .	18.43	6.93	.376	
	Fremont	Granitic sandy loam. . .	10.42	3.73	.358	
6	Arapaho . . .	Transported fine sandy loam	13.65	3.22	.236	
243	Leadville . .	Granitic loam . . .	45.40	7.60	.168	Do.
231	Battlement . .	Lava loam . . . . .	30.16	4.93	.163	Do.
232	do . . . . .	do . . . . .	21.83	3.73	.171	Do.
205	Bighorn . . .	Granitic sandy loam . . .	17.40	2.88	.166	Do.
206	do . . . . .	do . . . . .	13.63	2.17	.159	Do.
222	Gunnison . .	Limestone silt loam . . .	28.16	3.31	.118	Do.
221	do . . . . .	Limestone loam . . . . .	22.78	2.69	.118	Do.

In Table XXI there are presented all the wilting coefficients which have not been given in the two preceding tables, and for which the corresponding moisture equivalents are available. On examining the data, however, it is readily seen that in each group those tests which have been made without coating the soil with paraffin give much lower coefficients than those in which the soil was coated. This is as might be expected and, since the proportion of such tests is variable in the different groups, it seems best to discard all data obtained in this way. We then obtain the following averages:

Species.	Num- ber of tests	Mean moisture equivalent	Mean ratio wilting co- efficient to moisture equivalent.
		<i>Per cent.</i>	
Yellow pine. . . . .	16	8.42	0.3825
Lodgepole pine. . . . .	15	12.90	.3468
Douglas fir. . . . .	13	8.76	.3065
Engelmann spruce. . . . .	7	14.58	.3044

When allowance has been made for the fact, which is evidenced by the mean moisture equivalents, that three-fourths of the usable data for yellow pine and half of that for Douglas fir were obtained with granitic gravel, or sand soils, although these soils do not much effect the other two groups, it is definitely decided that wilting coefficients are much lower for Douglas fir than for yellow pine, probably somewhat lower for yellow pine than for lodgepole, and certainly lower for Douglas fir than for spruce.

With this confirmatory evidence, no hesitancy need be felt in placing these four species in the following approximate relationships:

Species.	Approximate mean ratio of wilting coefficients to moisture equivalents on common basis of soil qualities	Species	Approximate mean ratio of wilting coefficients to moisture equivalents on common basis of soil qualities.
Lodgepole pine.....	0.35	Engelmann spruce.....	0.32
Yellow pine.....	33	Douglas fir.....	.31

Certainly, from all the evidence available, the differences between the species are not any greater than here indicated. From all that we have found, it would probably be fair to say that in actual ability to stand drought, at least for the conditions existing in these pan tests, there is no essential difference between yellow pine, Engelmann spruce, and Douglas fir, the greater frailty and slow rooting of the spruce as compared with fir or pine being balanced by an actually stronger affinity of the spruce for any water within reach of its roots. On the other hand, lodgepole seems to stand out both as frail and slow-rooting, and with no compensating development of high sap density or osmotic pressure, so that it does succumb to drought much sooner than the others. In one test only, limber pine and bristlecone pine have shown themselves in practically the same class as yellow pine.

#### RESISTANCE TO EXCESSIVE HEAT

The sap densities observed in seedlings, and the relative rates of transpiration as apparently affected thereby, gave rise to the suggestion that there might be a specific difference in heat requirements based on this same set of internal conditions. While, on the one hand, the freely transpiring species of low sap density would seem to require a warm environment to counteract the cooling effect of this transpiration, on the other hand, the species of high sap density, which also seem to function more fully than others without full direct sunlight, appear to be always in danger of becoming overheated because, for some physical reason not fully explained, the heat absorbed is not so fully utilized in evaporation.

One thing which the close observation of seedlings in the wilting tests has made very plain is that at an early age all seedlings are very susceptible to injury just where the stems are in contact with the surface soil. At times it has seemed as though moisture absorbed by the roots might be extracted from the stems at this point, so blanched and shrunken do they become as soon as the surface of the soil becomes dry. On the other hand, it is perfectly evident that, as soon as the surface soil ceases to possess moisture to keep its temperature down, it may in sunlight easily become by far the warmest part of the environment. The measurement of the temperature at the warmest point is exceedingly difficult, but the showing of thermometers more or less submerged indicates that the soil surface not infrequently attains a temperature

of 160° F. It is, therefore, readily seen that in soils exposed to sunlight the injury resulting from drought at the surface may be indistinguishable from that due to superheating. Under ordinary circumstances the two injurious conditions will be inseparable.

The difficulty of determining the heat tolerance of seedlings at the point where they are commonly injured by heat is, because of the influence of moisture, very great. We have not been able to conceive a test of heat tolerance in the normal sense, except through the employment of sunlight or some other more powerful radiant energy. Every other possible plan of exposure to heat seems to have the objectionable result of injuring the foliage first, which rarely happens in nature, or of preventing normal evaporation with whatever protection that may afford.

Therefore, the only test<sup>\*</sup> that has been made to determine the relative tolerance of heat by forest-tree seedlings has been on this basis of obtaining as high temperatures as possible in sunlight, with the air to some extent artificially warmed. The actual temperatures attained were measured only so far as was possible by placing mercurial thermometers directly above the soil surface. The seedlings of each species were developed in several pans, each of which represented a different moisture content. Because of the fact that the largest amounts of soil moisture permitted almost no injury, the moisture contents were in several cases lowered before the test was completed, so that the record is considerably confused. From the data secured, however, the following conclusions, admittedly tentative, may be drawn.

1. Injury to seedlings from excessive heat is plainly greatest when the seedlings are youngest. This introduces a complicating factor in the test, because exposures to high temperatures were begun before germination was entirely completed and when, therefore, there was the most marked difference in ages. Engelmann spruce ordinarily germinates most promptly and spontaneously. Consequently, while there was marked early damage to this species, the fact that there were few later germinations left the species then immune for some time. Lodgepole pine exhibits just the opposite characteristics and effects.

2. Seedlings which survive a certain degree of exposure are not likely to be injured until the conditions become considerably more severe.

3. The ease with which any species may be injured increases very markedly as the moisture content of the soil decreases. With the lowest content, 3 per cent, which in this soil was appreciably above the wilting coefficient, it may be questioned whether the injury was not due to drought almost wholly, since between waterings the wilting coefficient of the soil was reached.

4. At all times the nature of the wilting was indistinguishable from that which occurs with similar seedlings when no excessive heat is involved. Consequently, it appears that wilting may be due as much to inability to supply transpiration losses as to the direct effects of the temperatures. The fact that no wilting was secured with 14 per cent moisture appears to bear out this idea, yet it must be remembered that this free moisture may have greatly reduced the temperature extremes of the surface soil. The fact that temperatures recorded just above the soil were not in excess of 135° F. further suggests that wilting was the result of transpiration losses rather than a direct temperature effect on the protoplasm.

<sup>\*</sup> Credit for the conduct of this test should be given to Forest Assistant J. Roeser, jr.

5. With this understanding of the situation we may say that in this test Engelmann spruce and lodgepole pine were most susceptible, while Douglas fir and yellow pine were about equally resistant. The factor which seems to control susceptibility is mainly structural rather than physiological—that is, it is the small mass of the spruce and lodgepole, and possibly their weak rooting, which causes them to stand out in contrast to yellow pine and Douglas fir under extreme drying conditions of relatively short duration.

This pairing of spruce with lodgepole suggests as strongly as do the high wilting coefficients for lodgepole the very poor ability of the latter to supply itself with water; but, in the light of the other facts secured, the same cause will not fully explain the behavior of spruce. It is believed it would be fairer in the case of spruce to say that high temperatures in direct sunlight create high internal temperatures and some direct heat injury. This hair-splitting distinction is necessary for the proper physiological interpretation which will agree with the other facts at hand. It may be added that the susceptibility of spruce to injury in sunlight has been very evident in many of the wilting coefficient tests.

#### EVIDENCE OF WINTERKILLING

Winterkilling of trees is generally recognized as the direct result of evaporation from the leaves or twigs at times when moisture can not be supplied to replace the loss, owing to a frozen condition of the soil. It is, of course, not confined to evergreen trees but may affect fruit trees, or even such hardy forest trees as honey locust, when devoid of foliage.

The conditions for winterkilling are usually provided by a very rapid rise in air temperatures and by wind which facilitates evaporation. The soil, of course, warming more slowly than the air, may not free its moisture for many hours after the beginning of the unseasonable air conditions. Likewise, if the tree stems have been thoroughly frozen, they may not be able to transport water until a great loss from the leaves and small twigs has occurred.

The conditions conducive to winterkilling are especially likely to be produced near the base of the Rockies from northern Colorado northward. The coniferous forests which are subject to this form of injury are therefore the low-lying yellow pine forests of the Black Hills and eastern Montana. Here the Chinook, a warm wind occurring at a season when the normal temperatures are below freezing, attains its most typical development.

A typical Chinook has not been noted within the locality of the present study. It has been shown, however, that in the Pikes Peak region the winds from January to March possess the powers of a modified Chinook. While they do not often bring extremely large rises in air temperatures, they are of high velocity, the air is dry, and the soils at all elevations, unless strongly isolated, are likely to be deeply frozen and remain so throughout the duration of the wind, which is often two or three days.

The Pikes Peak region therefore presents a good opportunity for the study of the relative resistance of the several species to this form of drought, for the desiccating influence is not confined to the low zone where only yellow pine occurs.

The present writer (*a*) has described in some detail the cumulative effects of winds occurring at the Fremont Station in January and March, 1916. It was shown that on a south exposure where yellow pine, Douglas

fir, and limber pine grow in a mixed stand, Douglas fir at first showed a more pronounced discoloration, but later the injury to yellow pine was seen to be much more severe, as only this species was defoliated. This injury was always much more pronounced on the west (windward) side of a tree, but it varied with different specimens, partly because the ground is strewn with large boulders which deflect the wind and also reflect sunlight. While in no case fatal (and even the general injury in the Black Hills in 1909 caused a very small percentage of deaths), this defoliation obviously must have a retarding effect on the growth of the whole tree. That the same kind of injury occurs at intervals of a few years, and that it hits "twice in the same spots," seems to be indicated by the one-sided development of most of the trees which were injured in 1916 (see Pl. 7, A). Buds and branches were rarely injured in this case, and new foliage appeared almost as early as on unaffected trees or parts.

In the nursery, where there was no snow to furnish protection during most of the winter, a better comparison of the species was possible because of the uniform conditions of soil and exposure. Yellow pine stock was damaged more than Douglas fir; Douglas fir far more than spruce. In fact, in only a few cases was spruce even discolored. With lodgepole the injury was usually confined to an exposed branch or leader, suggesting incomplete ripening of the previous season's growth.

This indicates, as do all other data, that spruce can bear drying to a greater degree than the other species, or at least that it resists the drying better, which comes to the same result. It is perhaps significant of the moisture-conserving adaptation of limber pine, which has been indicated by the transpiration tests, that there was no apparent injury to this species on the south slope where yellow pine was most plainly injured. It resisted wind-drying of this kind as well as any species. On the other hand, during the summer drought of 1917, limber pine was the only species showing injury to trees of large size.

#### SUMMARY

The relative qualities of the important forest trees of the Central Rocky Mountains, primarily from the standpoint of moisture relations, have been approached from five different angles. No one of these efforts has been free from errors, and no one would alone carry conviction, but the several results are corroborative with only insignificant exceptions. These comparisons of the species have been made on the basis of—

1. Measurements of the water used in relation to growth and leaf exposure of 3- to 9-year-old trees, under uniform conditions for all species.
2. Comparisons of sap density under uniform and varying growth conditions.
3. Measurements of the moisture of soils not available to young seedlings by direct comparisons of the species and also under varying conditions as to soil quality and atmospheric stresses.
4. Observations on fatality among seedlings under high temperature conditions.

5. Observations on the resistance to winter drought of specimens growing side by side, and as measured by the extent of injury to foliage.

It will have become apparent that there are several aspects of the moisture relations, that the several species studied do not always stand in the same relation one to the other, and that it is not even possible to state

that of the Rocky Mountain species one is distinctly more drought-resistant than the others. The moisture relations apparently vary much with the other environmental conditions, and it is perhaps the most important feature of this paper that a somewhat logical relationship has been shown to exist between moisture requirements and other requirements of each species.

We may, then, briefly outline the theory and at the same time observe how closely it applies to the behavior of each species under each of the situations that has been presented. We may take as our starting point the relative "tolerance to shade" of the several species, because this is a character which always has been quite closely observed by foresters and in which, empirically, rather definite lines have been drawn.

Briefly the physiological requirements appear to be related on this basis:

1. The species of greatest shade tolerance or greatest ability to make effective use of sunlight in photosynthesis will possess, other conditions remaining equal, after a period permitting accumulation the greatest amount of soluble carbohydrates in the leaves. In this fundamental respect we shall adhere, at least tentatively, to the classification indicated by the December, 1917, sap densities, as shown in Table XIII, placing spruce at the head of the list of our indigenous species, followed by Douglas fir, lodgepole, bristlecone, yellow, and limber pines.

2. The presence of considerable quantities of carbohydrates augmenting other solutes creates a dense sap, or solution, which does not evaporate so readily as a dilute solution. Because of the osmotic pressure exerted by a dense solution, there should at the same time be greater ability to extract water from the soil, though there is no evidence that at the end of the struggle one species tolerates appreciably greater drought than the others.

3. The presence of these solutes in large quantities is also, naturally, conducive to a high growth rate.

4. By restricting evaporation, the soluble carbohydrates may increase the net amount of the light energy available for photosynthesis, so that, whatever the original quality which made the plant effective, this quality is augmented by its own results.

5. By restricting the use of heat in evaporation, however, the dense cell sap may not only reduce the relative heat or light requirement of the species but may subject it to the danger of superheating. Of all the possible influences of the specific differences which give rise to the cell-sap differences, it is believed this is the most important ecologically and the most potent in its effect on the distribution of the species. If we assume distribution to be controlled primarily by this physiological factor, it becomes fairly simple to see how adjustments have been made to meet other conditions of the environment, principally in the form of structural adaptations, which differentiate the species beyond that difference which may arise from photosynthetic efficiency, and which may to a certain extent compensate for the physiological deficiencies.

If we accept the heat hypothesis as fundamental, we mean that each species will be limited in its distribution rather sharply by the maximum temperatures which it can tolerate (probably in the early seedling stage) and also limited in its growth by its minimum requirements, so that at a certain low temperature it is unable to compete with more highly developed species and hence loses its dominance in the forest. In the mountain forests, therefore, we should expect to find the six species zoned according to temperatures, in the order named just above.

This zonation holds, definitely, however, only for Engelmann spruce, Douglas fir, and yellow pine, which we have shown to be so equally developed as forest dominants that the fundamental physiological differences control all their relations. With the three more or less weedy pines there are, plainly, adaptations which are equally effective or more effective in controlling distribution. It is significant of the importance of high temperatures as absolute limitations that these three species are all found in higher and cooler zones than their physiological conditions necessitate.

Supplementing physiological characters, we may have stomatal reduction, thickened epidermis, or clustered leaves, all tending to reduce the absolute transpiration, but, while doing so, inevitably reducing either the intake of carbon dioxide or the effectiveness of sunlight so that photosynthesis and growth are reduced perhaps even more than is water loss. This seems to be the general line of protective development in the "weed" trees, limber pine and bristlecone pine, and to a lesser extent in lodgepole pine.

Again, resulting from gradual adjustment to the moisture conditions which accompany certain heat conditions, the forest trees have different root habits, or (shall we say?) are unequally stimulated to root development. It is believed that temporary stimulus has much to do with it, but inherited habit still more. Be that as it may, yellow pine and Douglas fir root much more vigorously at an early age than lodgepole pine or spruce. Almost as divergent are the germinating rates of the seed, lodgepole pine standing out as the most sluggish of the six species studied.

In the strictly physiological sense, spruce is undoubtedly the most highly developed of the indigenous species we have considered. This is evidenced by the sap densities which the trees show after long seasons of photosynthesis and by the amount of growth made in relation to the total amount of the water consumed.

In actual water consumed by a tree exposing a unit area to light (and wind) spruce is again the most economical, followed by Douglas fir, bristlecone, limber, yellow, and lodgepole pines. In this consideration, it is, almost without question, the special adaptations of the weed pines which put them down as only moderately extravagant, making them especially suited to exposed windy sites but wholly incapable of holding a permanent place in the forest. On the contrary, spruce maintains a moderate rate of transpiration under the driest conditions (so far as measured) for two reasons, namely, because it does not mechanically restrict losses but forges ahead with growth, and because when the water supply is low it is still more able than any of its competitors to supply its needs and is not so soon restricted either in transpiration or growth. These facts stand out very clearly. In this comparison we have placed Douglas fir next to spruce, believing that the actual position shown by Table XI is misleading, because the trees involved did not develop normally.

In resistance to winter-drying, limber pine with its peculiar structural development and spruce with its high physiological resistance have shown themselves about equally effective. Douglas fir and yellow pine follow with increasing weakness. Lodgepole pine shows greater resistance than would be expected, a fact which we shall not attempt to explain at present.

Considering the drought resistance of seedlings, through the wilting coefficients of a number of soils in which they have been compared, we find the same physiological properties evidently at work, though much



obscured by the relative sizes of the seedlings and their root developments. The seedlings of spruce and lodgepole pine are small and frail and in the first two or three months develop scarcely more than half the root produced by Douglas fir and yellow pine. As a result, even when carefully protected from excessively rapid water loss, lodgepole pine seedlings show far less drought-resistance than the others. Spruce seedlings, on the other hand, show quite as great resistance as those of pine or fir when not excessively insolated, and possibly a little more if the drought condition is approached every slowly. Limber and bristlecone pine seedlings, as meagerly observed, resist drought with the best of the others, no doubt because of a low rate of transpiration. In this connection the soil conditions leading up to wilting of seedlings should be borne in mind. Rarely is it possible for the roots to reach and extract all of the moisture which it would be physically possible for them to absorb. The completeness of this absorption depends very much on capillary movement in the soil. If the amount required by the seedling is small, this movement may supply the needs. Therefore, the rate of transpiration by the seedling is very important in determining, to a fine point, the degree of drought which it will resist.

In nature all possible rates of soil-drying are represented, dependent very much on the amount of insolation on the site and to some extent on the nature of the soil cover. The open south exposure will usually dry at the immediate surface very rapidly. Because of the lack of humus, however, the layer just below the surface may remain moderately moist so long as the quantity of water below is sufficient to maintain capillary movement. When that end is reached, the soil undoubtedly dries out very rapidly to a considerable depth. Even with the respite furnished by capillary movement, the whole process of drying, in continuously bright and dry weather, seems likely to be accomplished here sooner than in the contrasting site. This may be on bottoms or north exposures where the total moisture supply is sufficient to produce a closed stand, heavy shade, and the accumulation of humus. In this soil the surface litter and humus are rarely thoroughly wetted except during and immediately after the melting of snow. The more decomposed humus below, however, due to a lack of insolation and being protected by the surface litter, is rarely dry except after prolonged drought. It dries out slowly and steadily, however, both through the demands of the roots below and by direct evaporation. It follows that, since these demands in the aggregate are very large, such a soil may at unusual times, or possibly in the usual autumn drought, become extremely dry, especially so in the physiological sense, because of its high wilting coefficient.

On the one hand, then, we have the rapidly fluctuating moisture conditions of the well-insolated site, which, for the establishment of seedlings would appear to demand prompt germination and prompt deep rooting. Yellow pine is preeminently adapted to these conditions by reason of its large seeds, which produce large sturdy seedlings with a habit of immediate deep rooting. There is nothing in the evidence on this species to suggest conservatism in the use of water. Probably the extravagant use of water assists in protecting from excessive heat. Success is dependent on the roots reaching a layer of the soil which does not dry out dangerously through insolation. It follows logically that yellow pine can not attain success in the face of competition, either with the roots of established trees or with grasses and herbs whose use of the water would materially augment the drying of the surface layer.

The large moisture demands of yellow pine, with the normal amount of precipitation, can only be supplied in open stands, which first permit the safe establishment of the roots at a depth and later their extension into a large area of soil. This is of fundamental importance in the management of the species and explains the ultimate failure of one crop of seedlings after another in stands which are already moderately crowded or apparently fairly open.

Next in order on such sites we might expect to find Douglas fir, because it, also, produces a deep-rooted seedling. However, we should bear in mind that this species transpires less freely than yellow pine and hence may not be able to tolerate so much insolation. Extended observation shows that it will grow almost anywhere that yellow pine will grow, provided only the seedlings may have shade until they have passed the stage when most susceptible to heat injury. The fact that seedlings start in the shade of and in the densest root area of yellow pine trees shows that this species requires less moisture than yellow pine or, at least, that the moisture is not a controlling factor, and it is apparently for this reason that Douglas fir forms the climax forest except on the warmest yellow pine sites.

Limber pine and bristlecone pine are also, by germination and rooting habit, adapted to well-insolated sites. The sap density of bristlecone pine, however, is apparently considerably higher than that of limber pine, and therefore it succeeds better on cooler sites and on heavier soils. The physiological development of both species and their growth rates are so low that neither can hold a place in the forest in competition with spruce or Douglas fir.

On the other hand we have the poorly insolated sites, commonly described as "cool and moist," which are subject to comparatively slow and wide seasonal changes in their moisture conditions.

Spruce seedlings on account of their growth habit are able early in the season to penetrate the layer of loose organic matter which is in many seasons thoroughly wet only after snow melting. The small seeds germinate at a lower temperature than those of other species. Thereafter the roots show little stimulation to further growth. Even the dryness of the fall period does not appreciably stimulate root growth in the new seedlings, and it is believed that this is clear evidence of the ability of the species to extract water from the soil at a low degree of availability. Possibly because of their generally higher organization, spruce seedlings even prefer a low moisture content which results in greater concentration of the soil nutrients. In such a situation Douglas fir has no theoretical advantage over spruce except in case of a drought so prolonged as completely to dry out the soil layer in which the spruce roots are found. Then the deeper rooting of the fir should, apparently, count in its favor. But in the established forest this can hardly be a material advantage, considering the evenness and depth of the drying where the soil is well occupied by older roots.

The situation with regard to lodgepole is very different from that of our other forest trees, and the writer takes from the evidence the liberty of suggesting either that it is just approaching the physiological status of a full-fledged tree or that it is such a recent migrant to the Rocky Mountain region as to have failed by far to adapt its mode of growth to the poor moisture conditions usually found where its heat requirements are best satisfied. Some of the evidence on the latter point has been presented by the writer. (3). Clements (9) has classed lodgepole

as even more intolerant of shade than yellow pine, and a fairly low photosynthetic efficiency is clearly indicated in this paper. Yet, while evidently demanding a great deal of light as well as moisture during the growing period, it shows no such habit as that possessed by yellow pine, of prompt germination or deep rooting. It is evident, therefore, that it is adapted only to sites with a steady supply of moisture and demands more than can usually be supplied by either of the contrasting situations which have been described. It is probably for this reason that it reproduces readily only where competition is decidedly lacking, adheres to the higher elevations where moisture is more abundant but where its growth rate is surprisingly slow, and has not penetrated to the south where there is a very sharp contrast between the summer rainy period and the clear, dry weather of autumn. In its range there is, to be sure, a generally steady decrease in the precipitation from May or June onward, but this is usually so gradual as to permit a great degree of adjustment.

### CONCLUSION

In concluding this paper, it may be said that certain physiological relationships between the species which are of great importance, especially for a proper understanding of forest growth, have been brought out and tentatively established by approach from several angles, but that, from the standpoint of natural reproduction and in relation to all questions of natural distribution of the species, these relative physiological qualities are not shown to be more controlling than some adaptations of form and characteristics of behavior which may be adequately described only by the word "habit." Technical forestry or silviculture might be said to be based on the venerable concept that the several species of the forest vary in their demands for light or their tolerance of shade. This concept is not only not altered by the present results but is confirmed, and the relation of the photosynthetic capacity of a given species to its heat and moisture requirements is made much more clear and definite than it has appeared heretofore. Spruce is shown to be the most efficient of the species considered, not only because of its high photosynthetic capacity, but also because when this capacity is exercised the species automatically becomes economical in its use (by transpiration) of water and at least in this sense has low moisture requirements. At the same time it may be rendered sensitive to excessive temperatures. On the contrary, yellow pine, commonly thought of as very drought resistant, is found to require much light and heat and, with these, to use comparatively large amounts of water per tree of given size. Of course, the facts fit more closely with preconceived ideas when spruce stands are compared with pine stands, the much smaller number of individuals in the pine stand not only compensating for the high individual water use but being a most vital concomitant of this individual requirement. In other words, with the low moisture supply commonly available in the low elevations and warm situations (which alone insure proper development of the pine), wide root spread and an open stand are vitally necessary to insure the water supply of the individual tree. The practical importance of this fact in forest management should be very clear.

It will be seen, then, that these physiological relations principally clarify our conceptions of growth. Spruce is a better grower, a more efficient mechanism for growth, than Douglas fir, and fir more efficient

than pine. In forest planting, where use is made of trees which have been carefully nurtured beyond the stage of greatest susceptibility to heat and drought injury, this difference in the efficiency of our species, particularly the more effective use of water by spruce and fir, may be advantageously employed, the ranges of these species being artificially extended downward, without any injurious effects, while by natural reproduction such extension would be completely prohibited or would be very slow. With even greater care the economic loss resulting from the planting of any species in a situation too high or too cool, or in stands too dense for its proper development, must be avoided.

In short, when it is considered that any cutting of a forest, by admitting more light and creating higher temperatures in the surface soil and more rapid fluctuations of the moisture on which young seedlings are dependent, tends to encourage a species more "hardy" but of a lower order of development than the one which dominates the stand (not necessarily of lower technical value), it is not difficult to see that all forest management hinges on these relative physiological properties for which we have been groping. Finally, it may be said that all of the physiological relations are embodied in the now rather general conclusion of foresters that the highest returns can be had from forestry only when cutting is followed by planting.

This brings us to the original object of the present study, which has been to explain the existing natural forest types, to explain the distribution of the species. As has been pointed out, natural distribution is plainly influenced at present by habits and adaptations which have developed in each species and which to a considerable extent compensate or balance the more deeply embedded physiological qualities. Without again going into the details that have been brought out, we may illustrate to show how these developments affect natural distribution. In figure 5 an attempt is made to show the influence of these developments on distribution, in a broad way. It has been indicated that spruce may be very sensitive to high temperatures, but especially so at a very early age when the seedling is small and tender. In consequence of this weakness, which is a result of its most fundamental organic character, probably for ages no spruce seedling has been able to develop at a low elevation or on a very well-insolated site at a middle elevation. Such sites as are suitable in respect to insolation and heat must show almost invariably quite even moisture conditions and usually soils characterized by a surface layer of litter and humus. Consequently spruce has developed a rooting habit suited to these moisture and soil conditions—a relatively slow and feeble rooting habit which does not suffice for quick establishment under any other conditions. As a further consequence, spruce has evolved very small seeds, there being no need for large, sturdy seedlings or for deep rooting before the seedling may itself manufacture food. It is seen, then, without enumerating any other similar developments, that the species would have great difficulty in extending naturally to any sites other than the cool and moist ones on which it is commonly found. The common conception has been in error only to the extent of assuming that the essential feature of such sites is a large moisture supply. It is now fairly evident that the individual spruce tree does not require a large moisture supply even though this may insure the fullest development of the stand and, in view of this fact, that spruce may be used in planting where the moisture supply is relatively low.

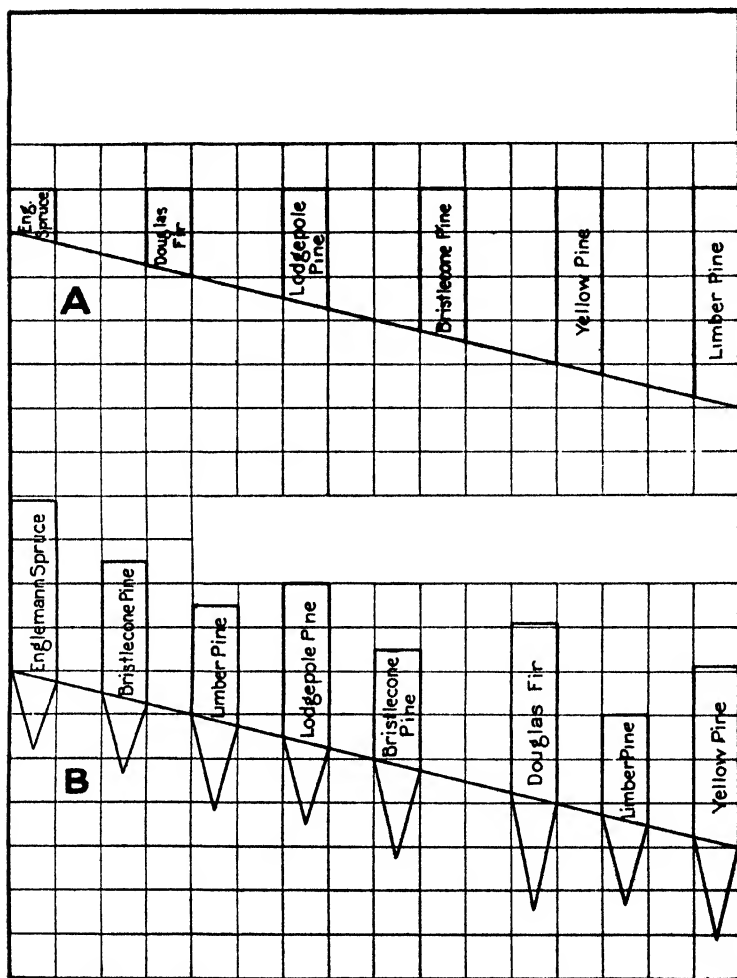


FIG. 5.—A, Theoretical zonation and relative heat requirements on the basis of photosynthetic capacity.  
B, Actual zonation and relative dominance as influenced by adaptations of roots and foliage.

It is to be hoped that this distinction between the temporary qualities of seedlings which acutely influence natural reproduction and extension of ranges and the more fundamental qualities of the species which later control growth reactions and economic values may be clearly held in mind, since it becomes increasingly apparent as time goes on that the factors controlling reproduction must be considered as almost independent of those controlling later growth.

In a succeeding paper on this subject it is hoped principally to show to what extent the environmental conditions of the different forest types differ and, in the light of what we have so far seen, to weigh carefully the importance of each condition so that those conditions which are really essential to the success of a given species may be clearly understood. The practical application of these facts in forest management may then be shown, it is hoped, in more definite terms.

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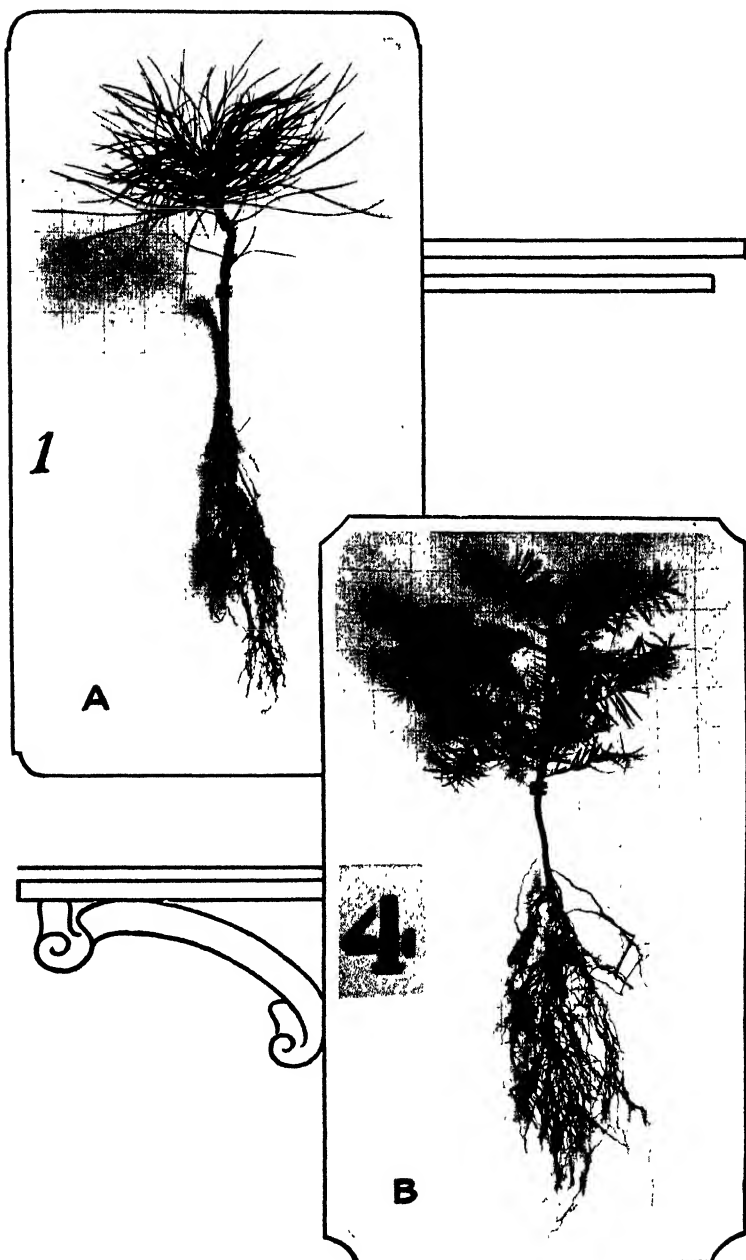


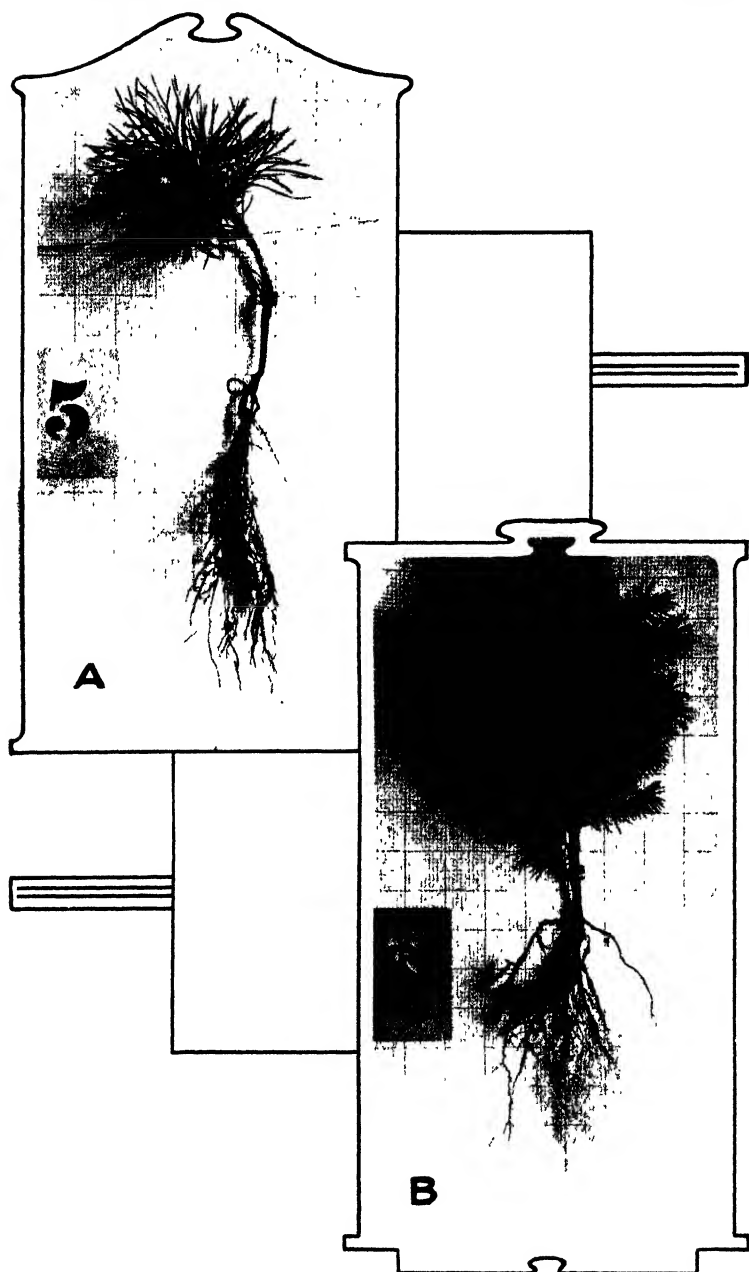


**PLATE 1**

**A.—Tree No. 1, yellow pine, 1917.**

**B.—Tree No. 4, Douglas fir, 1917.**





**PLATE 2**

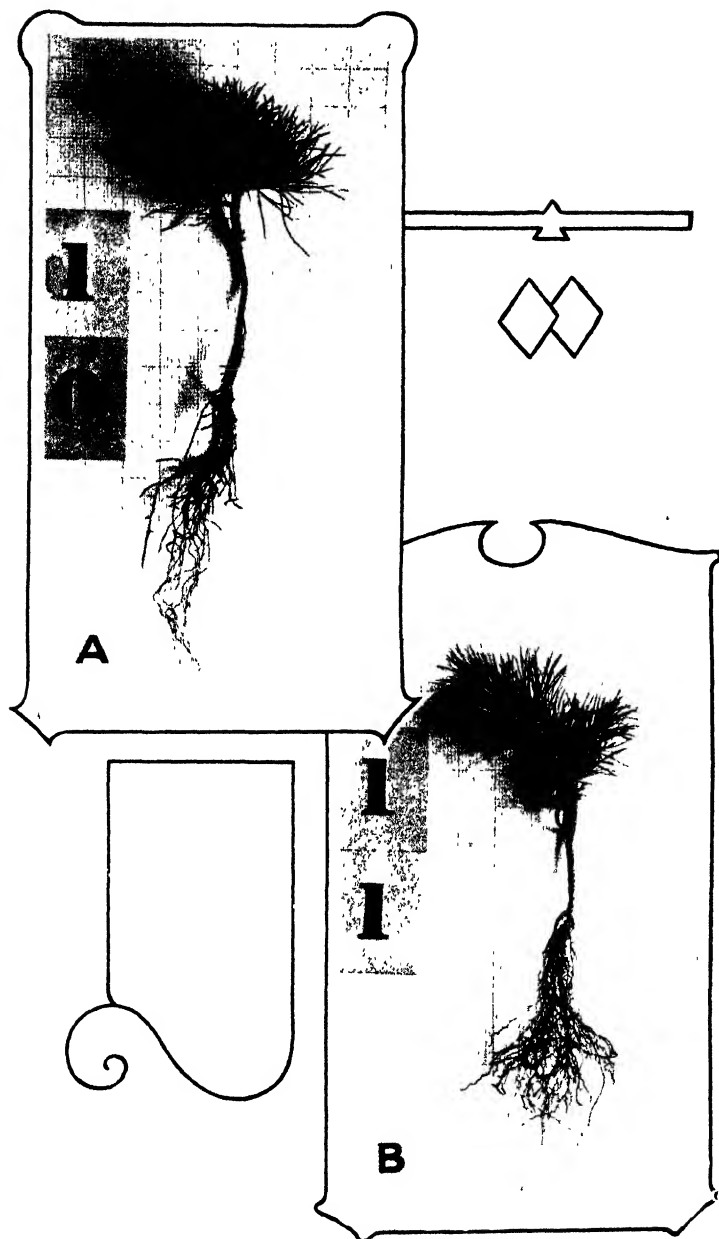
A.—Tree No. 5, lodgepole pine, 1917.

B.—Tree No. 8, Engelmann spruce, 1917.

PLATE 3

A.—Tree No. 10, limber pine, 1917.

B.—Tree No. 11, bristlecone pine, 1917.



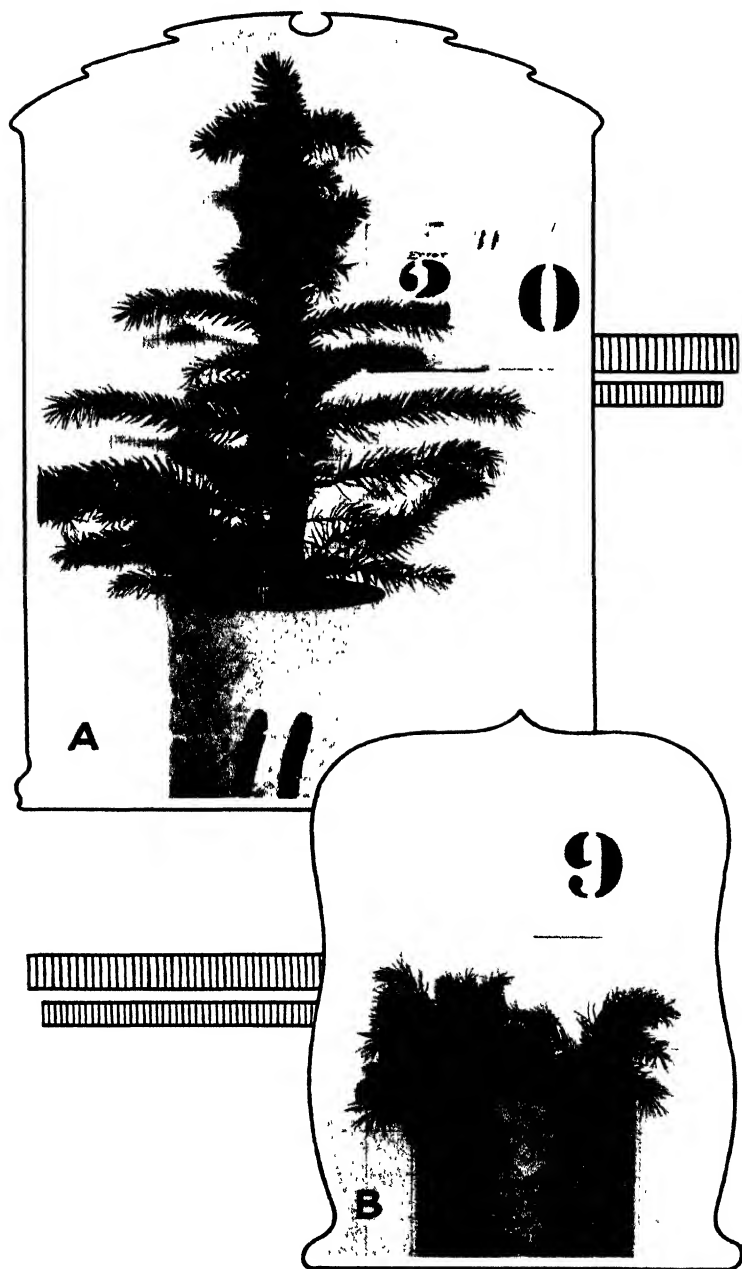


PLATE 4

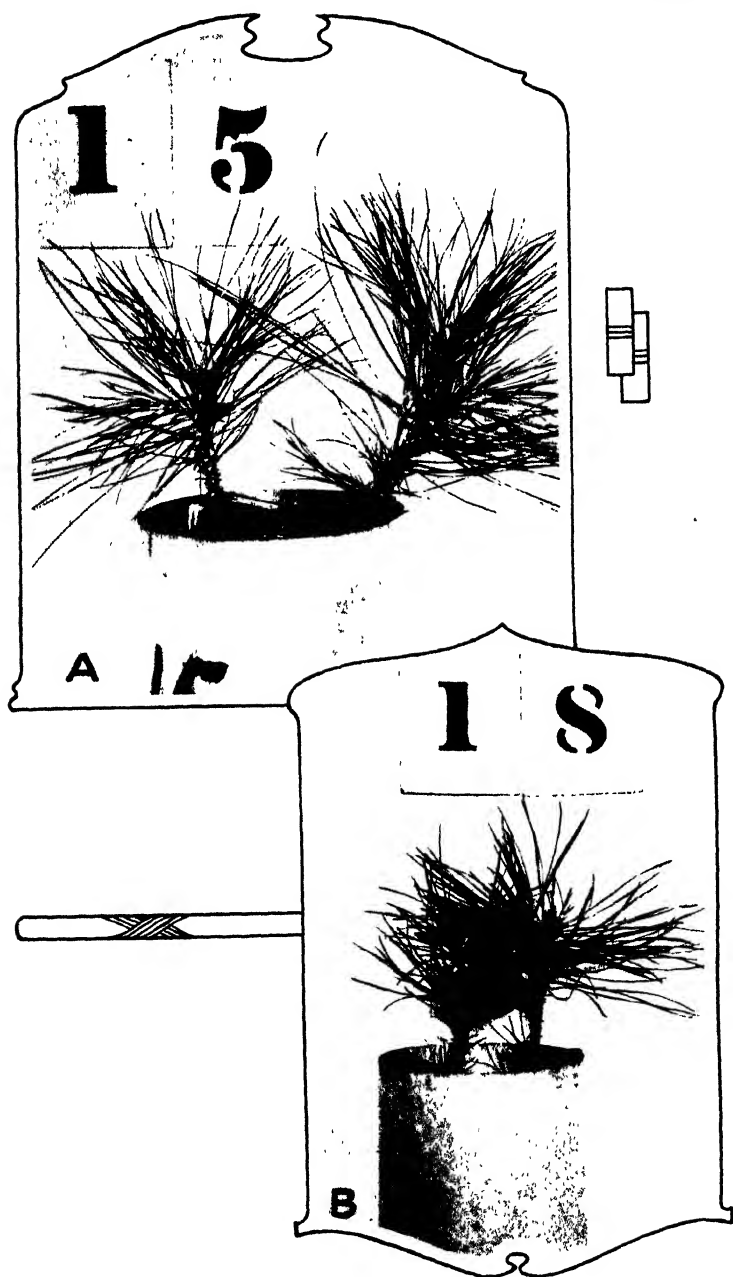
- A.—Large spruce, No. 11, in 1920 transpiration test.  
B.—Small spruces in Pot 9, 1920.

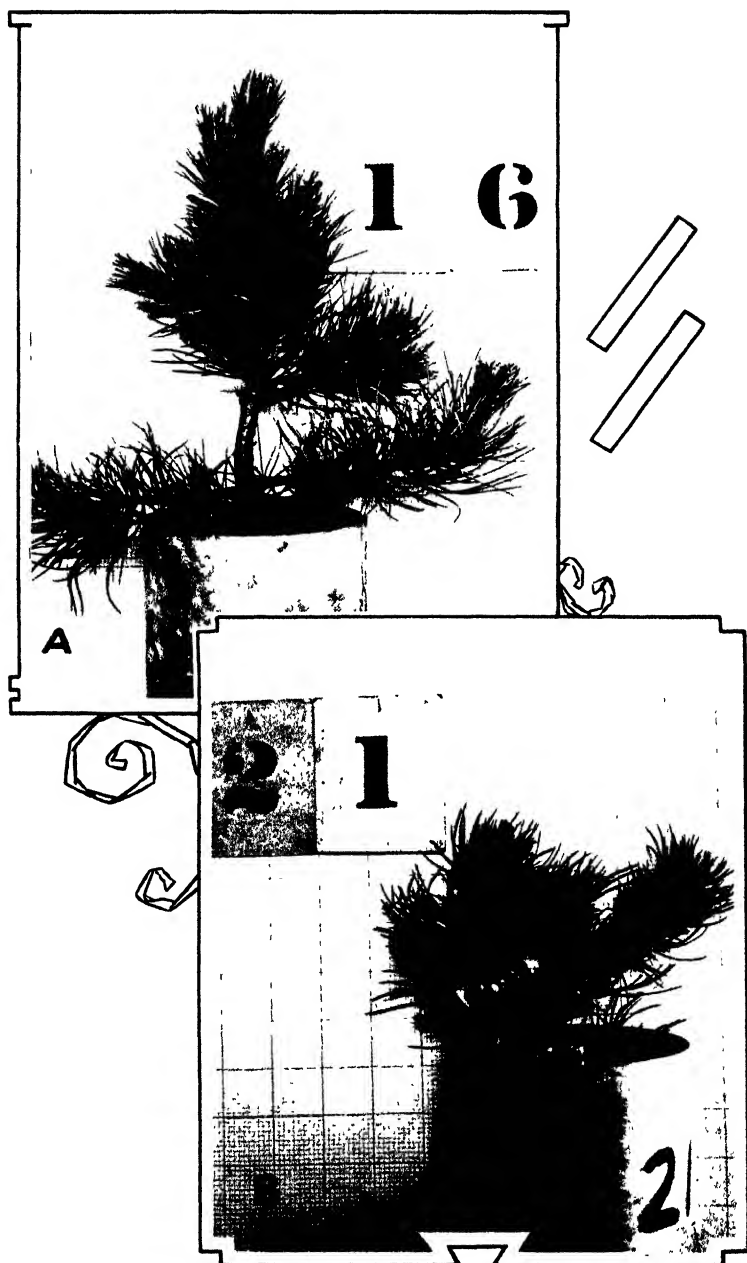


**PLATE 5**

**A.—Arizona yellow pine, Pot 15, 1926.**

**B.—Montana yellow pine, Pot 18, 1920**





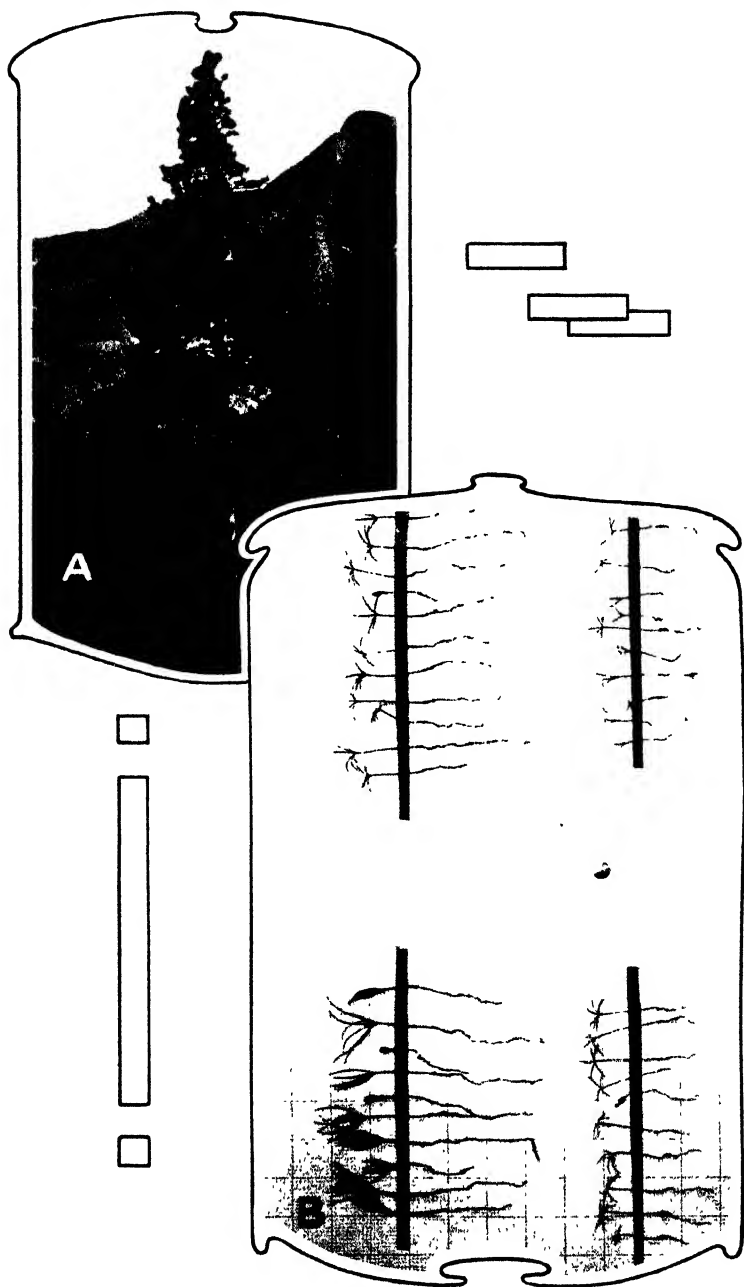
**PLATE 6**

- A.—Vigorous limber pine, Pot 16, 1920. Water requirement 773 units.**  
**B.—Sluggish limber pine, Pot 21, 1920. Water requirement 4,785 units.**

**PLATE 7**

**A.—Asymmetrical development of yellow pine, probably resulting from repeated winterkilling of limbs on the west side. (Looking south.) July 2, 1916.**

**B.—Relative root developments in moist sandy soil of seedlings 30 days after sowing.**





# A STUDY OF THE INTERNAL BROWNING OF THE YELLOW NEWTOWN APPLE<sup>1</sup>

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## INTRODUCTION

As a result of the heavy losses of Pajaro Valley apples during the early development of cold storage practices in California, considerable effort by the Bureau of Plant Industry of the United States Department of Agriculture has been directed toward determining the cause of this trouble and toward devising methods of overcoming it. In 1910 Stubenrauch (16)<sup>3</sup> indicated a relation between the browning and the temperature of storage, lower temperatures favoring its development. In the report of this bureau for 1920 (1) it was stated that there was no relation between the acidity of the fruit and the trouble and that as yet no definite cause could be assigned for this disease.

## OBJECTS OF THE INVESTIGATION

The present investigation is an attempt to determine the cause of internal browning, with special reference to (1) field conditions which are responsible for the susceptibility of the fruit and (2) the internal and external factors which are immediately responsible for its development in storage.

## DESCRIPTION OF INTERNAL BROWNING

Internal browning as it occurs in the Yellow Newtown apple is a nonparasitic storage disease of the large isodiametric cells of the pulp. In apples stored immediately, regardless of the time of harvest, at  $-1.1^{\circ}$  and  $0^{\circ}$  C. the browning generally becomes noticeable during the latter part of December, while in apples stored at higher temperatures its first appearance is proportionately later. The writer has not observed its occurrence in apples kept at temperatures of  $8.3^{\circ}$  or above.

In a cross-sectional view of the apple, the disease is first detectable in more or less elongated areas radiating outward from the central portion of the apple in the region opposite the basal end of the carpels. By cutting the apple in various planes it is apparent that the areas first browned lie adjacent to and radiating outward from the primary vascular bundles.

As the browning becomes more severe, it spreads most rapidly in the region of the secondary vascular bundles. In many specimens it

<sup>1</sup> Accepted for publication July 17, 1922. Submitted as a major thesis to the faculty of the Graduate Division of the University of California, May, 1921, in partial fulfillment of the requirements for the degree of doctor of philosophy.

<sup>2</sup> The writer wishes to express his thanks to Dr. J. C. Whitten, Dr. J. P. Bennett, and Prof. E. L. Overholser for counsel and suggestions during the progress of this investigation. The writer is also indebted to Mr. H. E. Jacob, a graduate student, for assistance in harvesting the fruit, in preparing it for storage, and in making the observations.

<sup>3</sup> Reference is made by number (italics) to "Literature cited." p. 184.



advances far toward the calyx end of the apples in this region before it penetrates more than several millimeters into the pulp laterally near the initial point of browning. Although the browning seems to be confined to the region immediately adjacent to the vascular system in its most rapid penetration of the apple tissue, the bundles themselves are slow to show the browning. The large cells adjacent to the bundles are the first to become discolored. Later the small cells bordering on the bundles are affected, and finally in the advanced stages of the disease the bundles themselves become brown. In its advanced stages the disease may spread to all portions of the pulp, constituting a condition comparable to the usual type of storage breakdown. In the latter condition, however, the browning begins at the calyx end of the apple, involving all of the tissue in its spread, and is accompanied by softening of the affected region.

It often happens that in the very advanced stages the browning spreads into the small thick-walled cells of the epidermis, thus giving the fruit an appearance of being scalded. In its less advanced condition, the disease, however, is detectable only by cutting into the fruit, the skin retaining its natural color and luster and the flesh remaining firm.

#### METHODS AND PLAN

The storage phases of this problem were carried out in a cold-storage plant consisting of six rooms and two large insulated boxes, making available the following temperatures: 0°, 2.2°, 5°, 8.3°, and 13.9° C. The temperatures were maintained within  $\pm 1^\circ$ , with the exception of the highest temperature, which varied from 12° to 14°. The humidity was practically constant in the different rooms, never varying more than 3 to 4 per cent.

The fruit was obtained from the Rogers Bros.<sup>4</sup> orchards, which are located about 1 mile east of Watsonville, Calif. The apples were packed and labeled under the respective trees from which they were picked. The fruit was not sorted, but represented tree-run apples, with the exception of the third picking in 1919, which was made up of grade "B" apples. The boxed apples were then expressed to Berkeley.

Three pickings were made each season. The earliest seasonal picking, 15 lots, was made just as the regular picking season was beginning. The second seasonal picking, a similar number of lots, came at about the middle of the normal picking season. The third picking was delayed until the close of the harvesting season. In each case a "lot" represented the fruit from a single tree.

The first two pickings for both seasons were stored the third or fourth day after picking. The storage of the last picking, however, was delayed approximately three weeks each season due to the slow shipment.

After arriving at the storage plant, the lots were divided into the required number of sublots for the several individual experiments. The sublots of approximately 80 specimens each, unless stated otherwise in connection with the separate tests, were stored in apple boxes. Sufficient space was always maintained between the boxes for normal ventilation. Not only were apples of the same lot used in each experiment and in the control, but the apples of each test were placed under as nearly identical conditions as possible.

<sup>4</sup> The writer is greatly obliged to Messrs. C. J. Rogers and Marion Rogers for their interest and hearty cooperation in the work.

As internal browning affects the flesh of the fruit, it was necessary to cut the apples in order to make observations upon their condition. The apples were cut perpendicularly to the axis of the core in a plane which passed approximately through the junction of the carpels with the stem. By cutting in this plane the browning was always detected, if present.

Observations upon the browning were made at monthly intervals beginning with February 2 for the 1919-20 season and with January 8 for the season of 1920-21. Twenty specimens from every subplot were cut at each of the four seasonal cuttings. By cutting several hundred apples on numerous occasions it was found that the error of observation in cutting only 20 specimens ranged from 2 to 5 per cent. When the results for the four seasonal cuttings were averaged, this error was reduced to 1 to 2 per cent. In recording the observations the following terms were used to designate the degree of browning:

1. **NORMAL.**—No browning apparent to the unaided eye; less than 0.1 per cent of cells affected. (Pl. 1, A.)

2. **TRACE BROWNING.**—Browning recognizable in the torus but not of sufficient intensity to lessen the market quality; 0.1 to 0.6 per cent of cells affected. (Pl. 1, B.)

3. **SLIGHT BROWNING.**—Browning in a sufficient degree of intensity to lessen the market quality of the fruit but not to such a degree as to make the apples objectionable for culinary purposes; 0.6 to 10 per cent of cells affected. (Pl. 1, C.)

4. **MODERATE BROWNING.**—Browning of such an intensity as to render the apples unsuitable for ordinary culinary purposes. At this stage of browning the tissue was more generally discolored throughout the torus, from 10 to 30 per cent of the cells being brown. (Pl. 1, D.)

5. **SEVERE BROWNING.**—This term refers to a degree of browning which upon cutting gave the apples an appearance of being rotten within. In these apples the structure of the tissue exhibited a marked degree of disintegration in all portions of the specimen; 30 per cent or more of the cells affected. (Pl. 1, E.)

#### PRESENTATION OF DATA

##### RELATIVE DEGREE OF INTERNAL BROWNING EXHIBITED BY YELLOW NEWTOWN APPLES GROWN IN THE PAJARO VALLEY AND ELSEWHERE

During this study apples were also obtained from other localities in California and from important districts in other States where this variety is successfully grown to determine whether or not the browning was confined solely to apples grown in this valley. All of the fruit was shipped to Berkeley by express and was then stored at 0° C. under the same conditions as the fruit from the Pajaro Valley.

The figures obtained in these tests indicate that Yellow Newtown apples generally are more or less susceptible to internal browning. The disease in apples from points other than the Pajaro Valley, however, has not been sufficiently severe to render it an economic problem.

The fact that all the apples showed browning would seem to indicate that either something peculiar to the variety makes it susceptible to browning or that the trouble lies in the regions in which it is at present most extensively grown. Both of these conditions appear to be more or less responsible for the browning. The fact that other varieties of apples grown in the same districts, with the exception of the Pajaro Valley, are immune to this disease would at least suggest that the Yellow New-

town exhibits a varietal characteristic of susceptibility to internal browning.

The effect of the region in which the fruit is grown upon its susceptibility to browning is indicated by the fact that several varieties of apples such as the Yellow Bellflower and the Red Pearmain, which normally show no tendency to brown, are susceptible to this disease when grown in the Pajaro Valley. These varieties do not brown when grown in any of the other Yellow Newtown districts. Furthermore, the Yellow Newtown, when grown in this valley is much more susceptible to internal browning than when grown elsewhere. The climatic conditions of the Pajaro Valley, therefore, seem to exert an influence upon the development of apples which has not been shown to occur elsewhere and which renders them susceptible to this disease.

#### RELATION OF TIME OF HARVESTING TO INTERNAL BROWNING

The importance of the time of harvesting of the fruit in the control of nonparasitic diseases of the apples has been stressed by Powell and Fulton (13), Brooks, Cooley, and Fisher (4, 5, 6), and others. Correspondence with cold-storage managers showed that some of them believe that internal browning is, at least in part, the result of picking the apples too green. The riper apples which have a higher sugar content, according to these men, are more resistant to browning.

An investigation of the effect of the time of harvesting upon internal browning was started in the season of 1919-20. Three pickings of fruit were made for both this and the 1920-21 seasons. The fruit of the first picking was "hard green" in maturity and of a solid green color; that of the second picking was "firm green" and signs of the yellow color were becoming evident; while the fruit of the last picking was "overripe" for harvesting and showed a considerable amount of yellow over the entire surface. The fruits for each lot were picked from all portions of the same trees at each of the pickings and were then stored under identical conditions at 0° and 2.2° C. The effect of the time of harvesting upon the severity and rate of browning are shown in Tables I and II.

The figures of Table I indicate that the later-picked fruit browned much more severely in every test. In the case of the apples stored at 2.2° C. during the season 1919-20, the actual figures in the table show little difference in the amount of browning. When the difference in the storage dates is taken into account, however, a considerable difference in favor of the earlier pickings becomes apparent. In the other cases the relation of time of harvest to the browning is obvious.

The figures of Table II indicate that the fruit of the last picking browned two and one-half times as rapidly as that of the second picking and the fruit of the second picking browned one and one-half times as rapidly as that picked at the beginning of the harvest season.

The sugar content of the fruit picked at the time of harvest September 26, October 16, and November 6 was 9.4, 10, and 11.4 per cent, respectively, which appears to indicate that a higher sugar content favors browning. By analyzing a large number of samples, however, it was shown that the sugar content does not influence the resistance or susceptibility of the fruit to browning. It might also be expected that the change in acidity of the fruit of the later picking, due to its more mature condition, and the subsequent prolonged storage would affect its resistance to browning. This was found not to be true. Although the titrable

acidity decreased with maturity and subsequent storage, the active acidity as indicated by the  $P_H$  value of the expressed juice remained practically constant. This possibly accounts for the fact that the decrease in total acidity does not influence the resistance of the fruit to browning.

TABLE I.—*Relation of time of harvesting of the fruit to internal browning*

SEASON OF 1919-20							
Storage temperature	Date of picking	Date of storage	Condition of fruit at end of storage period, approximately Apr. 1.				
			Normal	Trace.	Slight.	Moderate.	Severe.
° C.			Per cent.	Per cent	Per cent	Per cent	Per cent.
2.2 . . . . .	Sept. 28	Oct. 2	45	50	5	0	0
2.2 . . . . .	Oct. 18	22	50	45	5	0	0
2.2 . . . . .	Nov. 22	Dec. 8	45	45	10	0	0
0 . . . . .	Sept. 28	Oct. 2	40	20	15	15	10
0 . . . . .	Oct. 18	22	20	45	20	10	5
0 . . . . .	Nov. 22	Dec. 8	15	35	20	10	20

SEASON OF 1920-21							
2.2 . . . . .	Sept. 27	Sept. 30	75	10	5	10	0
2.2 . . . . .	Oct. 16	Oct. 19	60	35	5	0	0
2.2 . . . . .	Nov. 6	Nov. 26	5	45	30	20	0
0 . . . . .	Sept. 27	Sept. 30	30	40	20	10	0
0 . . . . .	Oct. 16	Oct. 19	20	45	25	10	0
0 . . . . .	Nov. 6	Nov. 26	0	30	45	25	0

TABLE II.—*The relation of time of harvesting of the fruit to the rate of development of browning*

Storage temperature	Date of picking.	Condition of fruit	Weeks in storage.	Market-able. <sup>1</sup>	Unmarket-able.
° C				Per cent.	Per cent.
0 . . . . .	Sept. 26	Hard green . . . . .	20	60	40
0 . . . . .	Oct. 16	Firm green . . . . .	15	60	40
0 . . . . .	Nov. 6	Overripe . . . . .	6	60	40

<sup>1</sup> Marketable fruit includes both normal and trace browned specimens.

# EFFECT OF TEMPERATURE UPON INTERNAL BROWNING

## STORAGE TEMPERATURE

At the time apple storage was introduced in California, investigations by Powell and Fulton (13) had brought the cold-storage men to the general belief that all apples could be stored most successfully at  $-5/9^{\circ}$  to  $0^{\circ}$  C. It was soon found, however, that great losses were incurred through the deterioration of Yellow Newtown apples of the Pajaro Valley at these temperatures. As a result of these losses investigations (16) were undertaken which brought about the storage of all the Pajaro Valley apples at  $2.2^{\circ}$ .

At the University of California, where apples of the Pajaro Valley have been stored at  $-1.1^{\circ}$ ,  $0^{\circ}$ , and  $2.2^{\circ}$  C. since 1916, it has been found that considerable browning occurs after the first of February, even in the fruit stored at  $2.2^{\circ}$ . Aside from the browning, however, these apples keep satisfactorily. It was, therefore, thought advisable to store apples at various temperatures above  $2.2^{\circ}$  in order to determine (1) the lowest temperature at which internal browning will not develop during the normal storage period and (2) whether or not the temperature which is sufficiently high to prevent internal browning is also sufficiently low for practical storage purposes.

During the season of 1919-20 the only other temperature available above  $2.2^{\circ}$  C. was that of room temperature. However, in 1920-21 apples were stored at  $5^{\circ}$ ,  $8.3^{\circ}$ ,  $13.9^{\circ}$ , and  $21^{\circ}$  in addition to the usual temperatures used for these apples. In the 1919-20 season 15 lots of the second picking were used in these tests, while in 1920-21 two lots of fruit from the same two trees for each of the first two pickings were used. Since all the apples browned in relatively the same proportions at each of the temperatures, only the averages for the second picking are given in Table III.

TABLE III.—*Effect of storage temperature upon internal browning*

SEASON 1919-20

Storage temperature.  °C.	Condition of fruit after 4 months' storage.				
	Normal.	Trace.	Slight.	Mod- erate.	Severe.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
-1.1 <sup>a</sup> .....	15	60	20	5	0
0.....	15	35	30	15	5
2.2.....	40	55	5	0	0
21.....	100	0	0	0	0

SEASON OF 1920-21

-1.1.....	15	35	25	20	5
2.2.....	40	35	20	5	0
5.....	95	5	0	0	0
8.3.....	100	0	0	0	0
13.9.....	100	0	0	0	0
21.....	100	0	0	0	0

<sup>a</sup> There was considerable freezing at  $-1.1^{\circ}$  C. in the early part of the storage season, which retarded the browning.

The figures of Table III show a definite relation between the amount of browning and the temperature of storage. The effect on browning of only a few degrees change in temperature is very striking. At  $0^{\circ}$  C., for instance, in the 1920-21 season, only 15 per cent of the fruit remained normal, while at  $2.2^{\circ}$  40 per cent was normal, and at  $5^{\circ}$  95 per cent of the fruit was normal. Browning did not occur in any of the fruit stored at a temperature of  $8.3^{\circ}$  or above.

It becomes manifest, therefore, that internal browning does not occur at a temperature a few degrees above that used in the commercial storage

of apples. Furthermore, the Yellow Newtown is known to be one of the best keeping apples, and it may be held quite satisfactorily in basement storage until May if well-matured, sound fruit is used. Thus it appears that where prompt storage under uniform conditions is possible the fruit can safely be held at temperatures sufficiently high to prevent browning without other forms of deterioration developing. In commercial practice, however, it would probably not be expedient to store apples above  $5^{\circ}\text{C}$ . It would, nevertheless, be advisable to store these apples at or just below this temperature.

The browning was not only increased in severity as the temperature decreased below  $5^{\circ}\text{C}$ . (as shown by the figures in Table III) but its development was also more rapid. This relation of temperature of storage, with the time of initial appearance and the subsequent development of the browning, is illustrated by the graphs in figure 1.

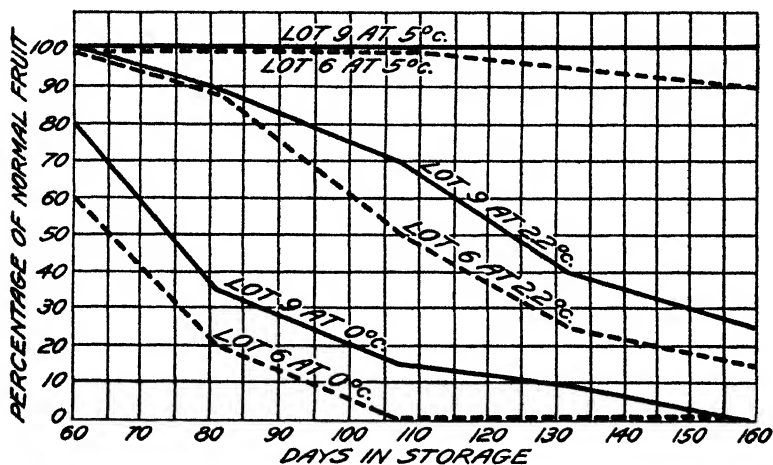


FIG. 1.—Effect of temperature upon the rate of development of internal browning.

#### ORCHARD TEMPERATURE

In the early investigations upon internal browning, letters, in the form of questionnaires, were sent to the leading fruit men of the Pajaro Valley. In reply to the question asking when the browning was most prevalent, many of the fruit growers attributed its occurrence to the cold, foggy weather which characterizes this valley during the latter part of July and the early part of August, just at the time the fruit is growing most rapidly.

With this observation as a basis, experiments were started in the spring of 1920 to determine the effect of orchard temperature and fog upon internal browning. During the first week of May, 1920, a tent of black cambric cloth was erected over a single average tree which bore a normal set of fruit. The sides of the tent came within about 8 feet of the ground. Thus, all the branches with fruit were shaded continuously. At the same time the tent was erected, 100 individual apples on an adjacent tree were placed in black cloth bags. A similar number of apples on adjoining trees were placed in black bags on the first of June and July, respectively.

Owing to the lack of necessary equipment, it was impossible to measure the exact effect of the tent and black bags upon the light intensity. Nevertheless, it is thought that the light exclusion as such is negligible, since it had little or no effect upon the amount of browning as shown by the figures of Table V. The number of foggy days might, of course, exert an indirect effect by influencing the temperature.

The effect of the tent and black bag upon the temperature, however, was very striking. The mean daily temperature at the core of the bagged fruit, as indicated by self-recording thermometers, was from 2.5° to 5.5° C. higher than that of apples normally exposed. In the case of the tented tree, a lower mean temperature was maintained by the shading and lack of free circulation of the air. Here the temperature, as recorded by accurately regulated thermograph instruments, was found to be from 2° to 4.5° lower than that for a similar position in an adjacent untented tree.

The fruit from the tented tree, the bagged and the normally exposed fruit from the same trees, and fruit from two adjacent trees for control were harvested at the first pickings of the 1920-21 season. All the lots and controls were stored under identical conditions at 0° C. The results of these experiments are given in Table IV. As the fruit of the two pickings behaved similarly, only the averages are given in the table.

TABLE IV.—*Effect of orchard temperature upon internal browning*

Treatment	Condition of fruit at end of storage period, Apr. 1.				
	Normal	Trace	Slight	Moderate.	Severe.
Fruit placed in black bags where temperature of apples was approximately 4° C. above that of fruit in open . . . . .	Per cent. 95	Per cent. 5	Per cent. 0	Per cent. 0	Per cent. 0
Fruit normally exposed . . . . .	30	35	25	10	0
Fruit from under tent where temperature was about 3.5° C. below that in open . . . . .	5	15	40	35	5

The figures of Table IV show a very definite relationship between the orchard temperature and internal browning. A daily mean temperature of 4° C. above the normal temperature of the orchard practically prevented the browning, while a mean temperature of 3.5° below the normal mean orchard temperature greatly increased the amount of browning over that which occurred in the normally grown fruit.

This relation between orchard temperature and the amount of browning becomes more impressive when the temperature records of this valley are compared, for the years of severe and of moderate or no browning for this region, with the temperature records of other districts where this variety of apple grows satisfactorily and where the browning is not a problem. The graphs in figure 2 represent the mean temperature for June, July, August, and September for the Pajaro Valley, Calif.; Albemarle County, Va.; and Rogue River Valley, Oreg. If the record for the Pajaro Valley is considered, it will be seen that in 1908 and 1914, years in which very heavy losses through internal browning occurred, the mean temperature for these four months was very low. For 1909, 1910, 1915, and 1916, when the mean temperature for these

growing months was nearly normal, the severity of the browning was less. In 1912, 1913, 1917, and 1918, years of higher mean temperature for the months of rapid growth, there was no browning in the commercial storage plants. Comparing the temperature records of the Pajaro Val, ley with those of Rogue River Valley, Oreg., and Albemarle County, Va., it is found that these regions have a mean temperature of 2.8° and 6.2° C. higher, respectively, than that of the Pajaro Valley.

Results which further confirm this relation of orchard temperature to the browning were obtained by collecting fruit from well-exposed and from shaded portions of the tree. Two lots of fruit were collected from the upper southwest periphery of two trees where the fruit received the maximum effect of the sun's rays, while two other lots were picked from the lower north, shaded portion of the same trees where the fruit was continuously in the shade. These lots were stored side by side at 0° C. The results of this experiment are given in Table V.

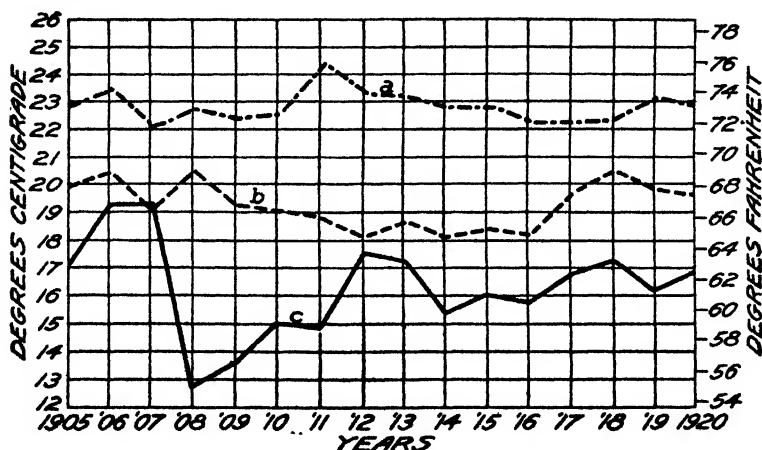


FIG. 1.—Mean daily temperature for June, July, August, and September from 1905 to 1920 for (a) Albemarle County, Va., (b) Rogue River Valley, Oreg., and (c) Pajaro Valley, Calif. (From climatological data reports, U S Dept. of Agr. Weather Bureau 1905 to 1920.)

TABLE V.—Effect of exposure of the fruit on the tree upon internal browning

Location of fruit on tree.	Condition of fruit at end of storage period.				
	Normal	Trace.	Slight	Moderate	Severe.
	Per cent	Per cent	Per cent.	Per cent	Per cent
Well exposed, on upper southwest periphery of the tree	35	55	10	0	0
Shaded, on lower north side of the tree	15	50	25	10	0

These figures show that the quantity of normal fruit from the well-exposed portions was 20 per cent greater than that obtained from the shaded portions of the same trees. While no records were taken with regard to the temperature of the fruit at the different exposures, it is safe



to assume that the mean daily temperature of the well-exposed fruit was somewhat higher than that from the shaded side of the tree.

Further evidence which seems to show the definite relation of low orchard temperature to the development of internal browning in this variety of apple is brought out in the results obtained from the fruit received from New York State. One lot of fruit was obtained from the Cornell Agricultural Experiment Station, where the mean summer temperature is only slightly more than 1° C. above that of the Pajaro Valley and where cloudy or rainy weather frequently prevails during the growing season. The other lot was obtained from northern part of Dutchess County, N. Y., where the mean summer temperature is approximately 3.2° higher than that of the Pajaro Valley and where, as a rule, there is an absence of cloudy or rainy weather during the growing season. After express shipment to Berkeley, Calif., these lots were stored under identical conditions at 0°. The figures in Table VI give the results of this test.

TABLE VI.—*Effect of orchard temperatures upon the susceptibility of New York Yellow Newtown apples to internal browning*

Source of the fruit	Weeks in storage	Condition of fruit at end of storage period.				
		Normal.	Trace	Slight.	Moderate.	Severe.
Cornell Agricultural Experiment Station, N. Y. . . . .	14	Per cent. 5	Per cent. 50	Per cent. 15	Per cent. 0	Per cent. 0
Dutchess County, N. Y. . . . .	13	100	0	0	0	0

The quantity of normal fruit obtained from Dutchess County was 95 per cent greater than that from the Cornell Station. This points very strongly to the fact that orchard temperature during the season of rapid growth is an important factor in the development of fruit which is susceptible or resistant to internal browning.

The results obtained on the effect of orchard temperature upon the subsequent susceptibility of the fruit to browning point to the possibility that the mean temperature for the growing season in the Pajaro Valley hovers around the lower limit for the normal development of this variety of apple.

#### RELATION OF ESSENTIAL OILS TO INTERNAL BROWNING

Before taking up a discussion of the data with regard to the relation of essential oils to internal browning, a few references will be made to previous work on the effect of essential oils and allied substances upon the cell.

Dixon and Atkins (8) have shown that anaesthetics increase the permeability of the plasma membrane, for the cell sap is readily expressed after their application. When applied for this purpose, however, the anaesthetics were toxic and their effect irreversible. Since a distinctive mark of an anaesthetic is the reversibility of its action, Osterhout (12) made measurements upon tissues to determine whether the increase in permeability, usually observed to follow their application, is due to the anaesthetics or to toxins. He concludes that the anaesthetics produce

a decrease in the permeability which is reversible and the subsequent increase in permeability is due to the accumulation of toxic substances as a result of the action of the anaesthetics. In 1910 Armstrong (2) and his co-workers showed that, under the influence of anaesthetics and certain other substances which they called hormones, reactions occur in the cells which indicate that the enzymes and their substrates were brought into contact. Among the results of this mixing of the enzymes and substrates, as observed by these workers, was an oxidation which resulted in pigmentation. These workers also state that these phenomena are constantly taking place in the plant but that under normal conditions their products are passed off before they become injurious. Under abnormal conditions, however, they may accumulate in sufficient amount to greatly hinder the activities of the tissues and eventually to cause the death of the cells. Giglioli (9) found that essential oils markedly influence the movements of water, enzymes, and soluble substance through the cell membrane. Later, Giglioli (10) also demonstrated that the enzymes could be removed from yeast cells by rendering them permeable with essential oils.

During the past year Power and Chestnut (14) have isolated the essential oils of the apple. They have shown conclusively that essential oils are being produced continuously by the apple in sufficient quantities to be detected. In 1919 Brooks, Cooley, and Fisher (5, 6, 7) found that apple-scald, a nonparasitic storage disease which is generally confined to the surface of the fruit, was apparently due to volatile substances which are produced by the fruit when held for some time under the more or less abnormal conditions of storage. In substantiating this contention, they present data which show that the disease is reduced to a minimum by removing these volatile substances from the fruit by air circulation or by storing the fruit in wax or oil wrappers that are known to be good absorbents of essential oils.

After making observations upon the appearance of the fruit in internal browning and in advanced stages of apple-scald, the writer became convinced from the firmness of the tissue and the way in which these diseases spread into the flesh of the fruit that there is a similarity between these two storage diseases. Histological examinations of affected tissues further emphasized the analogy which exists between these diseases. For the histological studies, sections of the apple torus were cut by means of a freezing microtome. The sections were dropped directly from the razor into acidified absolute alcohol which fixed them and prevented any additional browning. They were then passed through xylol and mounted in Canada balsam without staining. The examination of a large number of cells brought out a very striking similarity between browning and scald in the progress of the discoloration in the tissues as well as in the individual cell. In the tissue there was no regularity in the spread of the disease from one cell to the other, since isolated cells showing browning were always found to be scattered among the normal cells near the regions of scald or browning. In the cells in which the progress of the browning could be followed it was found to be identical in the two diseases. The browning started at the periphery of the cell and from there spread to all parts of the cell along the more concentrated strands or areas of cytoplasm. The discoloration was, as a rule, more intense in the region of the nucleus which is near the surface in the apple cells. Plasmolysis accompanies the advanced stages in browning, until, in the very severe stages, the

protoplast occupies only a small fraction of the cell. The cell wall remains unchanged. As a result of this apparent similarity between these diseases the writer carried out experiments upon the control of internal browning which had proved effective in reducing the amount of apple-scald.

#### AIR MOVEMENT AS A PREVENTIVE OF INTERNAL BROWNING

Two sublots of apples of the 1919 and 1920 seasons were stored in slat boxes, one box being wrapped in the ordinary manner while the other was stored without wrapping. These apples were then ventilated by a fan for 10 to 20 minutes twice each week. In the season of 1920-21, apples were also placed in sealed containers which were fitted with tubes for pulling air through with a filter pump. Thirty-three apples of the same lot were placed in each container, while a similar number in ordinary storage served as a control. The results of these experiments are given in Table VII.

TABLE VII—Effect of air movement upon the development of internal browning

Treatment	Season	Storage temperatures	Condition of fruit at end of storage period				
			Normal	Trace	Slight	Moderate	Severe
		° C	Per cent	Per cent	Per cent	Per cent	Per cent
Apples in common storage	1919-20	2.2	35	60	5	0	0
	1920-21	0	5	75	15	5	0
Apples in slat box, wrapped, ventilated 10 to 20 minutes twice each week.	1919-20	2.2	45	50	5	0	0
	1920-21	0	60	40	0	0	0
Apples in slat box, not wrapped, ventilated 10 to 20 minutes twice each week.	1919-20	2.2	100	0	0	0	0
	1920-21	0	75	25	0	0	0
Apples wrapped but not ventilated with filter pump <sup>1</sup>	1920-21	0	0	5	10	50	35
Apples wrapped, air drawn through slowly with filter pump <sup>1</sup>	1920-21	0	70	25	5	0	0
Apples not wrapped, air drawn through slowly with filter pump <sup>1</sup>	1920-21	0	70	30	0	0	0

<sup>1</sup> Apples sealed in cans with arrangement for slow renewal of air

The figures of Table VII indicate that where ventilation was employed there was a great decrease in the amount of fruit that exhibited the disease. The figures also show a very definite relation between the effectiveness of the ventilation and the severity of browning, for in every case the wrapped fruit showed more browning than did that which was not wrapped. This definite reduction by ventilation in the amount of browning would seem to indicate that the trouble is favored by the accumulation of deleterious substances which were removed by both the intermittent and the slow but continuous air movement.

## GAS ABSORBENTS AS AGENCIES IN THE PREVENTION OF INTERNAL BROWNING

Several sublots of the 1919 and 1920 crops of apples, picked October 18, were stored at 0°C. in oil wrappers which were known to be good absorbents of gases. For this purpose commercial 10 by 10 inch apple wrappers were saturated with the given wax or oil. The number of treatments included and the results of these tests are given in Table VIII.

TABLE VIII.—*Effect of gas absorbents upon the development of internal browning of apples stored at 0°C.*

Treatment.	Condition of fruit at end of storage period									
	Season of 1919-20					Season of 1920-21				
	Normal	Trace	Slight	Moderate	Severe	Normal	Trace	Slight	Moderate	Severe
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Cocoa-butter wrapper	10	45	20	5	0	50	40	10	0	0
Olive-oil wrapper	40	60	0	0	0	55	45	0	0	0
Wesson-oil wrapper	50	50	0	0	0					
Mazola-oil wrapper	50	45	5	0	0					
Vaseline wrapper...	70	25	5	0	0	45	55	0	0	0
Lard-tallow wrapper						15	75	10	0	0
Paraffin wrapper						5	80	15	0	0
Control, common wrapper						5	85	10	0	0
Control, no wrapper						5	65	20	10	0
Average for oil wrappers	48	46	6	0	0	34	57	7	0	0
Average for the controls	20	25	25	20	10	5	75	15	5	0

These data indicate that the amount of browning can be reduced by employing agents which absorb essential oils or emanating gases. Since all the tests as well as the controls were stored in identical boxes and under as nearly as possible the same conditions in the storage rooms, the beneficial effect of the oil wrappers must lie in their ability to prevent the accumulation of injurious substances. There was a reduction of about 30 per cent in the number of specimens showing browning in each case.

At the time the fruit picked October 18, 1920, was stored, 10 portions of 33 specimens each of lot "A" were placed in sealed containers with various wrappers as listed in the table. This was thought to be a more accurate method of determining the effectiveness with which certain absorbents control the disease. It would seem logical to assume that there was always a considerable supply of esters or other deleterious material in the storage room; hence the wrappers in the open boxes should absorb these as readily, if not more so, than the substances from the individual specimens which were wrapped, thereby dissipating their ability to function as active absorbents. In the sealed containers, however, the esters to be absorbed were more nearly confined to those produced by the inclosed fruit, and this should materially lengthen the period during which the oils in the wrappers would act as absorbing agents. The results of these tests are given in Table IX.

TABLE IX.—Effect of gas absorbents upon the development of internal browning

Treatment. <sup>1</sup>	Condition of fruit after 11 weeks at 0° C.				
	Normal.	Trace	Slight.	Mod- erate.	Severe.
	Per cent	Per cent	Per cent.	Per cent	Per cent.
Ordinary commercial wrappers.....	0	5	0	60	35
Wrappers impregnated with cocoa butter .....	65	35	0	0	0
Wrappers dusted with animal charcoal .....	45	50	5	0	0
Wrappers dusted with silica powder .....	0	5	5	45	45
Wrappers impregnated with olive oil .....	55	45	0	0	0
Wrappers impregnated with paraffin .....	25	65	10	0	0
Wrappers impregnated with lard-tallow mixture..	30	60	10	0	0
Wrappers impregnated with vaseline .....	65	35	0	0	0
Commercial wrappers, with 225 cc. concentrated potassium hydroxid in bottom of container.....	80	20	0	0	0
Commercial wrappers, with 225 gr. soda lime in bottom of container .....	0	5	5	50	40

<sup>1</sup> Apples sealed in cans with arrangement for slow renewal of air

The data in Table IX show a very striking relation between the prevention of the browning and gas absorbents. In these tests 95 per cent of the treated fruit was marketable, as compared to only 5 per cent of that of the controls. The figures also indicate a definite relation between the capacity of the various absorbents for taking up esters and the prevention of the disease. Paraffin, which according to Gilde-meister and Hoffman (11) has an absorbing power of approximately one-half that of the other substances, showed the least prevention of browning. The poor showing made by the tallow-lard mixture was possibly due to the fact that it became rancid before the experiment was little more than started.

All the tests with gas absorbents, as well as those with air circulation, seem to indicate that internal browning is caused by the accumulation of certain materials in the nature of essential oils or other volatile substances.

A very perplexing question which then arises is that of the appearance of scald on the surface while internal browning develops in the flesh of the fruit. In an attempt to answer this question, the writer placed fruit under optimum conditions for the development of both diseases. That is, apples were placed in stagnant air at a temperature which favors the development of the disease. The results of these tests were very interesting. In every case it was found that the scald and browning developed almost simultaneously. The scald rapidly developed into what is termed "deep scald," while the browning diffused outward at a similar rate from the points of initial appearance about the vascular bundles. In many specimens where the diseases were retarded more on one side of the fruit than on the other, a very interesting comparison of their spread could be made. The generally observed appearance of scald on the surface without the internal browning and the reverse condition would then seem to indicate that these two regions of the fruit are most susceptible to the essential oils, or that these substances accumulate more pronouncedly in these than in any other region of the apple. The disease seemingly appears first in that region which is most

susceptible. In the Yellow Newtown, apparently, the region of greatest susceptibility is in the flesh, while in those varieties that scald readily, it is at the surface.

If internal browning and apple-scald are caused by the accumulation of essential oils, which can be removed by ventilation or by absorption, the question arises as to why the preventive action of ventilation and absorbents is less marked in the control of internal browning than in apple-scald. This difference in the effectiveness of the prevention is undoubtedly due to the fact that apple-scald is the result of the accumulation of deleterious substances on the surface of the fruit where the absorbent can be brought close to them. Internal browning, on the other hand, is caused by an accumulation of the deleterious substances deep in the tissues, from whence they can be removed only by the very slow process of reducing their concentration at the surface, thereby inducing them to diffuse outward.

#### INCREASE IN PERMEABILITY PRIOR TO THE APPEARANCE OF INTERNAL BROWNING

If internal browning is due to the action of some deleterious substance which tends to accumulate in the flesh of the apple under storage conditions, there should be some evidence of its action before the browning actually occurs. By this it is meant certain alterations will occur in the cells which will permit the browning to take place. Possibly the most important, as well as the most probable, change which could take place is that of altering the permeability.

The changes in permeability were determined by measuring the resistance offered by the tissue to the passage of an electric current. Electrodes for this purpose were patterned after those used by Small (15). The electrodes were mounted so that they stood  $2\frac{1}{2}$  mm. apart and in such a manner that they would be pressed  $\frac{1}{2}$  cm. into the tissue. The measurements were made by the Kohlrausch method. The fruit was cut as in making the observations upon the browning and the electrodes were then pressed into the various regions in which measurements were to be made. The readings were made by bringing the minimum point to the same position on the bridge each time. These readings, therefore, indicate only the relative resistance of the different regions in the fruits. (For the information of some readers it may not be amiss to state that a decrease in the resistance offered to the passage of an electric current is interpreted to mean an increase in the permeability of the cells.)

Since it was impossible to obtain the above apparatus until late in the season, the results which are recorded in Table X give only one stage in the permeability changes that occur during the course of an entire storage season. All the measurements, with the exception of those given under tests No. 4, 5, and 11, were made in tissue which showed no browning.

TABLE X.—*Permeability at the surface and in the region of browning*

(Expressed in ohms resistance)

Test No	Fruit tested	Majority of readings.		Extremes of variation.	
		In region of browning	At the surface.	In region of browning.	At the surface
1	Apples of lots 6 and 9, stored at 8.3° C.	1,600 to 1,800	1,600 to 1,800	1,500 to 2,000..	1,500 to 2,000.
2	Same as (1), but stored at 5° C.	2,300 to 2,500	2,600 to 2,800	2,000 to 2,600..	2,300 to 2,800.
3	Same as (1), but stored at 0° C. (These lots browned at 0° C.)	1,100 to 1,300	2,300 to 2,500	1,100 to 1,800	2,300 to 2,800.
4	Same as (3), but reading taken in trace brown tissue.	900 to 1,200	2,300 to 2,400	800 to 1,300...	2,000 to 2,800.
5	Same as (3), but reading made in severely brown tissue	600 to 900	2,200 to 2,400	500 to 1,100	1,900 to 2,600.
6	Fruit from black bags, very resistant to browning at 0° C.	2,200 to 2,600	2,600 to 2,900	2,000 to 2,900	2,300 to 2,900.
7	Apples from same tree as bagged fruit, very susceptible to browning at 0° C.	1,000 to 1,200	2,300 to 2,500	1,000 to 1,600	2,000 to 2,700.
8	Virginia apples, very resistant to browning at 0° C.	2,500 to 2,700	2,600 to 2,800	2,300 to 2,800	2,500 to 2,800
9	Santa Cruz Mountain apples, very resistant to browning at 0° C.	2,200 to 2,500	2,500 to 2,800	1,700 to 2,500	2,300 to 2,800.
10	Lots 3, 5, and 8, very susceptible to browning at 0° C.	1,000 to 1,400	2,400 to 2,800	1,000 to 2,000	1,700 to 2,800.
11	Same as (10), but readings made in moderately browned tissue	700 to 900	2,300 to 2,600	500 to 1,100	1,600 to 2,800.

The figures of Table X show very definitely that there is a change in the permeability. At 8.3° C., where browning does not develop, there was an increase in permeability. These apples, however, were rapidly approaching storage breakdown, due to overripening at this relatively high temperature. The permeability had not increased in the apples stored at 5° where the ripening process was much slower and where the fruit remained free from browning. This was also true of the fruit at 0° which was resistant to the browning. In the fruit stored at 0° that which was susceptible to the browning, there was a greater increase in permeability in the interior of the specimens than occurred in the fruit stored at 8.3°. The fruit at 0°, nevertheless, was not approaching storage breakdown but incipient browning. In the specimens showing browning the permeability continued to increase with the advance in the severity of the disease. Therefore these data seem to indicate that, just prior to and accompanying the end of the storage life of these apples, there is a very marked increase in permeability, regardless of whether deterioration is brought about by storage breakdown or by internal browning.

## INCREASE IN PERMEABILITY DUE TO ESSENTIAL OILS

If internal browning be due to the accumulation of essential oils or similar deleterious substances which change the permeability, these oils should also increase the permeability when applied to the surface of the cut fruit. In order to test this property of these substances, several essential oils in great dilution were applied to the fruit about the electrodes of the conductivity apparatus. After the initial resistance was taken a drop of the solution was applied around the electrodes. Then the reading of the resistance was made every 5 minutes for a period of 20 minutes. The results of these tests were recorded in Table XI.

TABLE XI.—*Effect of essential oils upon the permeability of apple tissue*

[Expressed in ohms resistance]

Treatment	The initial resistance.	Resistance at various intervals after the application of the essential oils			
		After 5 minutes.	After 10 minutes.	After 15 minutes.	After 20 minutes.
Control, no treatment (current on continuously).....	2,900	2,800	2,800	2,400	2,200
One drop of 0.1 per cent solution of amyl acetate.....	2,800	1,600	800	550	350
One drop of saturated solution of amyl valerate.....	2,900	1,600	1,000	800	600
One drop of 1 per cent solution of acetaldehyde.....	2,900	1,600	1,000	750	500
One drop of 0.001 per cent solution of amyl acetate.....	2,900	2,000	1,100	900	600
One drop of 0.001 per cent solution of acetaldehyde.....	2,700	2,100	1,200	900	800
Water about the electrodes.....	2,800	2,400	2,200	2,100	2,000

The figures in Table XI show conclusively that essential oils increase the permeability when brought in contact with fruit tissues. These data indicate also that only a very small accumulation of essential oils might be sufficient to increase the permeability of apple cells, allowing the oxidase and substrates to come in contact, thus resulting in the browning. This is especially true when the greatest dilutions of the substances used in these tests are compared with the normal essential oil content of some apples as given by Power and Chesnut (14).

#### GENERAL DISCUSSION

In view of the relation of the browning with lower mean temperatures, it seems possible that the more severe browning of the mature fruit was due to exposure to the lower temperature which prevailed during the latter part of the harvesting season. The fruit of the second picking was exposed to a mean temperature of about 2.5° C. below the mean temperature of the growing season for three weeks after the fruit of the first picking was harvested. The fruit picked November 22 was exposed for six to eight weeks to the influence of a mean daily temperature of 2.5° to 8° below that prevailing at the time and before the first picking was made. As a whole, these data point to the possibility that the low temperature favors those conditions within the fruit which are necessary for the development of browning. This weakness in the fruit, if it can be considered as such, may be due to an abnormal development of the protoplasmic structure of the apples or to an accumulation of some deleterious substance which brings about a more rapid cessation in the normal functioning of these structures in storage. This seems probable since there was no appreciable difference between the resistant fruit and the fruit very susceptible to browning in constituents such as sugars, acid, and the  $P_H$  value of the expressed juice, which, it is generally believed, might influence a reaction of this sort through their effect upon the equilibria within the cells.



The accumulation of essential oils or similar deleterious substances also seems to be rather closely linked with the weakness in these apples that shows up in storage. This is indicated by the great reduction in the amount of browning that is brought about through the employment of air circulation or the impregnation of the wrappers with good absorbents for these substances. It has been further demonstrated that the permeability of the cells, which is the most probable change that might precede this browning or similar reactions, is increased very rapidly by essential oils when applied even in great dilution to the apple tissue. It was also found that there was an increase in permeability prior to and accompanying the death of the cells in the apple regardless of whether death was due to the usual type of storage breakdown (which is the result of overripening) or to internal browning.

The data obtained upon the relationship of temperature and the accumulation of essential oils or similar volatile substances to the browning, although not conclusive, point to several possibilities concerning the cause of this disease. When these apples are grown at a mean temperature as low as that of the growing season of the Pajaro Valley, they may fail to develop normally, hence when they are placed in storage the flesh of the fruit exhibits a susceptibility to injury through the action of the volatile emanation of the apple. This is indicated by the behavior of the fruit from different regions as well as by that from under the tent and in the black bags. This lower temperature may not only affect the development of the fruit but also apparently influence the production or accumulation of the volatile substances which are immediately responsible for the browning. This becomes apparent when the great difference in the amount of browning which developed at the several storage temperatures is taken into account. Seemingly there is a greater production of these substances at the lower temperatures, or otherwise they must accumulate more rapidly in those regions of the torus that are first to show the browning.

The reduction in the development of the browning by the use of gas absorbents also indicates that these volatile substances are present in injurious amounts at the lower temperatures under the ordinary conditions of storage. The more rapid accumulations of the deleterious substances may seem the more probable way of accounting for the injurious amount of these substances when the decrease in their volatility and the decrease in the permeability of the tissue at the lower temperatures is considered. However it is likely that there is also a greater production of these substances under the somewhat abnormal conditions of the lower temperatures of storage.

The nature of the process which results in the browning becomes of interest in connection with the preceding possibilities as to the cause of this trouble. Plausible explanations of this process could possibly be ascribed to an increase in the permeability of the protoplasm which permits the enzymes and their substrates to mix, or to the inactivation of some inhibiting substance. These changes might be brought about by the accumulation of certain substances such as the essential oils which are produced by the apple in storage and which apparently have a toxic effect upon the protoplasm of the cells.

In the normal cells the enzymes are prevented from acting upon their substrates by inhibitors or through lack of contact due to the possibly impermeable nature of the phase surface of the protoplasm. When the phase arrangements in the protoplasm, however, are acted upon

by toxins, these substances are no longer prevented from coming into contact. Similarly, the toxins may act upon the inhibitors to inactivate them. As a result of this liberation, the tannins of the apple cells may be oxidized to a brown by the oxidase which is also present in the mature fruit. It has been indicated by Bartholomew (3) that changes similar to these precede the blackening of the tissue in blackheart of potatoes. This explanation of coloration based upon a change in the permeability is also supported by the fact that before browning occurs there is a great increase in permeability of the cells as indicated by the conductivity measurements.

#### SUMMARY

(1) Internal browning is a nonparasitic disease of the large isodiametric cells of the flesh of the fruit.

(2) All Yellow Newtown apples, regardless of where grown, may in some years be susceptible to internal browning. This variety when grown under conditions prevailing in the Pajaro Valley has proved to be much more susceptible to this disease than when grown in other fruit regions.

(3) The later the fruit was picked, the greater the amount of browning which occurred in storage.

(4) No browning occurred even after four to six months storage in any of the fruit stored at 8.3°C. or above.

(5) The browning at the end of six months' storage at 5°C. was very limited and mild, and did not detract from the commercial value of the fruit.

(6) At 2.2°C. approximately 70 per cent of the apples showed browning by April 1, figures for each of the three seasons show. During the season 1920-21 which was average with regard to browning, only 50 per cent of the fruit stored at this temperature was marketable on April 1, 1921.

(7) At 0°C. practically all the fruit showed browning before April 1, each season. Only 20 per cent of the fruit stored at this temperature was marketable on April 1, 1921.

(8) A lowering by about 3°C. of the mean orchard temperature during the growing season, by tenting or shading a tree, greatly increased the susceptibility of the fruit to browning. After 4½ months' storage at 0°C. the fruit of the tented trees showed 25 per cent less of normal specimens than that of adjacent trees which were naturally exposed.

(9) An increase of 4°C. in the mean orchard temperature, by bagging individual apples in black cloth during the growing months, markedly increased the resistance of the fruit to this disease. The bagged apples showed 66 per cent more of normal specimens after 4½ months' storage at 0°C. than the naturally exposed fruit of the same trees.

(10) The browning was greatly reduced by ventilating the fruit.

(11) The browning was also reduced by impregnating the wrappers with oils and waxes which were good absorbents of essential oils.

(12) By measuring the electrical resistance of the apple tissue, it was found that there was an increase in permeability prior to the end of the storage life of the apple, regardless of whether death was due to the usual storage breakdown or to internal browning.

(13) It was demonstrated that essential oils when applied to the apple tissue even in great dilution rapidly increase its permeability.

(14) The data indicate that internal browning is due to the accumulation of essential oils or similar deleterious substances which are produced by the apples in storage. This shows that internal browning and apple-scald are quite closely related with respect to cause.

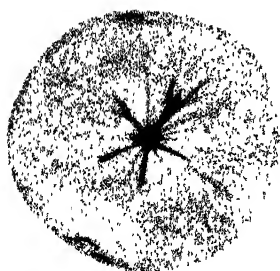
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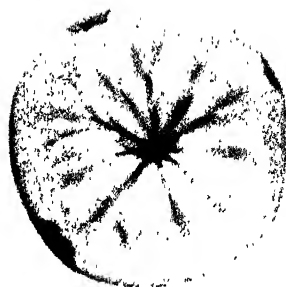


PLATE 1

- A.—A normal apple.
- B.—Average condition of trace browning.
- C.—Average condition of slight browning.
- D.—Average condition of moderate browning.
- E.—Average condition of severe browning.



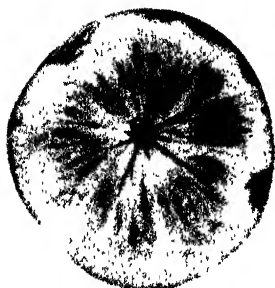
A



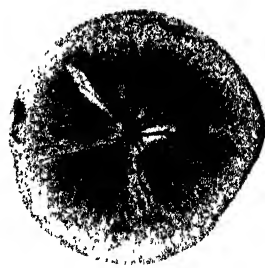
B



C



D



E



# ON THE USE OF CALCIUM CARBONATE IN NITROGEN FIXATION EXPERIMENTS<sup>1</sup>

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In reviewing the literature on nitrogen fixation by soil bacteria one is impressed with the great variety of media that have been employed by different investigators. Many of these media, while generally satisfactory, have not proved entirely so when employed by other investigators with slightly different environmental conditions.

It is not the purpose of this paper to enter into any discussion of the relative merits of different media, but rather to call attention to a frequent fundamental difference and its possible bearing upon the success attending their use. This difference is the presence or absence of calcium carbonate.

Winogradsky (9)<sup>2</sup> in his original experiments on nitrogen fixation by anaerobic bacteria used a dilute solution of the various salts necessary to furnish the elements essential to growth. To this was added a simple sugar as a source of energy and an excess of calcium carbonate to neutralize the acids formed from the sugar. Winogradsky's medium has been almost universally adopted for anaerobic nitrogen-fixing experiments.

Beijerinck (2), studying the aerobic *Azotobacter* group of nitrogen-fixing bacteria, found that a 0.02 per cent solution of  $K_2HPO_4$  in "Leitungswasser," to which was added a source of energy, furnished the necessary conditions for good growth of these organisms. Either the water or the inoculum must have furnished the other essential elements in sufficient quantity. The reaction of this medium was unaltered, the statement being made that—

Die Nährlösung reagiert durch das  $K_2HPO_4$  schwach alkalisch  
and that—

Die Alkalisch Reaction ist für den versuch günstig.

Beijerinck preferred mannite or a salt of propionic acid as a source of energy because—

Mannit kann nur schwierig und langsam, Propionate durchaus nicht der Butter-säuregärung anheimfallen.

Beijerinck further states that—

Die Produkte die Oxydation sind Kohlensäure und Wasser.

However, he realized that in impure cultures from soil, organic acids might be formed. Beijerinck failed to secure appreciable fixation of nitrogen by pure cultures.

Lipman (3) began a study of the *Azotobacter* group of nitrogen-fixing organisms shortly after Beijerinck. He demonstrated that Beijerinck's failure to secure fixation in pure cultures was due to the unfavorable re-

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 189-190



action of his media. The media adopted by Lipman was composed of tap water 1,000 cc., mannite 15 gm.,  $K_2HPO_4$  0.5 gm.,  $MgSO_4$  0.2 gm., a drop of 10 per cent solution of ferric chlorid, and enough sodium hydroxid to make the solution slightly alkaline to phenolphthalein. Lipman showed that within certain limits the quantity of nitrogen fixed was proportional to the quantity of sodium hydroxid added. He further demonstrated that the addition of  $CaCO_3$ , even to the medium made alkaline to phenolphthalein with sodium hydroxid, rendered it more favorable for nitrogen fixation. With regard to the influence of calcium carbonate Lipman (4) says:

It is clear therefore, that the presence of calcium carbonate stimulated growth either directly by furnishing calcium, or indirectly by making available more phosphorus, sulphur, and magnesium.

However, Lipman apparently lost sight of the value of calcium carbonate, for he did not recommend its use in his laboratory guide (5).

Ashby (1), apparently following the lead of Lipman, proposed that the acidity arising from the phosphate be neutralized with sodium hydroxid and in addition an excess of calcium carbonate be added. The medium proposed by Ashby has been more widely used than any other. It has the following composition: Distilled water 1,000 cc., mannite 12 or 20 gm.,  $MgSO_4$  0.2 gm.,  $KH_2PO_4$  0.2 gm.,  $NaCl$  0.2 gm.,  $CaSO_4$  0.1 gm., and 0.5 gm. of  $CaCO_3$  to each culture of 75 or 100 c. c. The phosphate is dissolved separately in a little water and made neutral to phenolphthalein with sodium hydroxid. Ashby found that the presence of calcium carbonate favored nitrogen fixation and that *Azotobacter* would sometimes develop in the presence of calcium carbonate but would not form a film if the carbonate were left out. Ashby also found that magnesium carbonate was even more efficacious than calcium carbonate, thus showing that calcium was not the essential constituent. Other investigators have since shown that other basic compounds can be substituted for calcium carbonate.

In addition to the three types of media just mentioned Löhnis and his students have made rather extensive use of soil extract to which was added  $K_2HPO_4$  and mannite or some other simple organic source of energy. In comparing the fixation of nitrogen in a medium of this type with and without the addition of calcium carbonate Löhnis and Pillai (7) found, as a rule, slightly greater fixation where the carbonate was added. However, Löhnis failed to adopt the use of calcium carbonate generally in his work, or to recommend its use in his laboratory guide (6).

It remained for Stoklasa (8) to produce the necessary evidence for a correct understanding of the function of calcium carbonate in nitrogen-fixation experiments by demonstrating quantitatively the formation of organic acids in cultures of *Azotobacter*. A survey of the accumulated literature on the subject will show, however, that many investigators failed to realize the significance of Stoklasa's results.

Practically all investigators agree that a neutral or alkaline reaction is desirable, if not essential, for the best development of nitrogen-fixing organisms. In most work some effort is made to adjust the medium to an alkaline reaction before inoculating, but in many cases no effort is made to maintain such a reaction. Even the influence of the inoculum upon the initial reaction has usually not been taken into consideration.

So far as the writer is aware no one has ever reported a detrimental effect upon nitrogen fixation from the presence of calcium carbonate in

the medium, even when present in large excess. This is an important consideration, however, since if it has no toxic effect upon the organisms it may be added in excess of initial requirements and thereby tend to maintain a favorable reaction throughout the experiment.

There are a number of isolated experiments such as those cited above showing the effect of calcium carbonate upon the growth of nitrogen-fixing organisms. In the course of some experiments, conducted by the writer, in which the relative growth of *Azotobacter* from a large number of different soils was compared there was an opportunity to observe the effects of  $\text{CaCO}_3$  on nitrogen fixation.

## METHODS

The medium employed had the following composition: Mannite 20 gm.,  $\text{K}_2\text{HPO}_4$  0.2 gm.,  $\text{Mg SO}_4$  0.2 gm.,  $\text{NaCl}$  0.5 gm.,  $\text{FeCl}_3$  trace, and water 1,000 cc. Two-hundredths gm. of  $\text{CaCl}_2$  was sometimes added, although in most cases because of the high calcium content of local soils the  $\text{CaCl}_2$  is not essential and was without effect. In those tests to which no  $\text{CaCO}_3$  was added the medium was always rendered slightly alkaline to phenolphthalein with sodium hydroxid. When  $\text{CaCO}_3$  was to be added the medium was sometimes first rendered slightly alkaline to phenolphthalein and at other times the reaction was unaltered prior to the addition of the  $\text{CaCO}_3$ . Fifty cc. of the medium were placed in 300-cc. Erlenmeyer flasks, and the  $\text{CaCO}_3$  was added in the form of sterile powder just prior to inoculation. No superiority is claimed for this medium over a score of others that might have been used. Obviously a medium with as variable composition as that containing tap water or soil extract would be unsuited for comparative work that must extend over a long period of time.

Samples were always set up in duplicate and total nitrogen determinations made on the whole sample. Total nitrogen determinations were also made in duplicate upon the inoculum. The inoculum consisted of 10 cc. of the supernatant suspension prepared by shaking one part of soil (50 to 100 gm.) with two parts of water and allowing to settle long enough for the larger particles to sink to the bottom. It is believed that such an inoculum is more representative of a mass of soil than 5 gm. of soil, and at the same time the quantity of solid material added is not sufficient to interfere in the least with total nitrogen determinations. Incubation was at room temperature for three weeks. In estimating the quantity of nitrogen fixed that present in the inoculum was deducted. Only the average of check determinations were recorded.

Frequent examinations of the cultures were made, both macroscopically and microscopically to ascertain whether *Azotobacter* were present. If *Azotobacter* make an appreciable growth it can usually be recognized by the appearance of the film. A microscopic examination of an unstained mount from such a film will reveal an unmistakable picture. A film is sometimes encountered which at certain stages in its development resembles quite closely an *Azotobacter* film, which, under the microscope, is found to be composed almost entirely of filamentous fungi, no organisms typical of *Azotobacter* being observed. In other instances nontypical films examined under the microscope would be found to be composed largely of fruiting fungi, the spores of which often closely resembled individual cells of *Azotobacter*.

If these examinations failed to reveal organisms morphologically similar to *Azotobacter* they were regarded as absent. Owing to the above-mentioned complex conditions it is quite possible that *Azotobacter* were sometimes reported present when in reality they were absent and vice versa. The end to be gained did not seem to justify the large amount of time that would be necessary to isolate and identify *Azotobacter* from the various soils. It is believed that if *Azotobacter* are not present in a soil in sufficient numbers and vigor to develop unmistakable evidence of their presence by the methods just described, for practical purposes they may as well be absent.

## RESULTS

Several hundred samples of soil from Kansas and other States have been examined by the methods described above. The following is a comparison of the average quantity of nitrogen fixed by 200 soils.

All samples .....	5.87 mgm.
Presence of $\text{CaCO}_3$ .....	7.10 mgm.
Absence of $\text{CaCO}_3$ .....	4.60 mgm.
<i>Azotobacter</i> film formed .....	7.70 mgm.
No <i>Azotobacter</i> film formed .....	4.10 mgm.

There were only two samples that failed to show some nitrogen fixation, and both of these were in media containing no  $\text{CaCO}_3$ .

When calcium carbonate was added to the medium an *Azotobacter* film was formed from 117 samples, or 58 per cent of the soils. The average quantity of nitrogen fixed in these was 8.1 mgm. The average quantity of nitrogen fixed in the 83 samples having no *Azotobacter* film was 5.7 mgm.

When no calcium carbonate was added to the medium an *Azotobacter* film was formed from 75 samples, or 38 per cent. These had fixed on the average 7.1 mgm. of nitrogen. One hundred and twenty-four samples, or 62 per cent, produced no *Azotobacter* film, and the average nitrogen fixed for these was 3.1 mgm.

Twenty-seven samples, or 14 per cent of all soils examined, fixed more nitrogen in the samples to which no  $\text{CaCO}_3$  was added, while 173 samples, or 86 per cent, fixed larger quantities of nitrogen in those samples receiving an addition of  $\text{CaCO}_3$ . The microscope revealed *Azotobacter* in cultures from 130 samples, or 65 per cent of all. No *Azotobacter* were observed in cultures from 70 samples, or 35 per cent of all. Some nitrogen fixation took place in practically all samples inoculated regardless of the source of the soil.

There were 12 samples containing *Azotobacter* or organisms resembling *Azotobacter* that failed to form an *Azotobacter* film. The average nitrogen fixed by these 12 soils where  $\text{CaCO}_3$  was added was 6.2 mgm. The average in the absence of  $\text{CaCO}_3$  was 3.1 mgm. This is 0.5 mgm. higher than the average fixed by those giving no film when  $\text{CaCO}_3$  was added and exactly the same as those giving no film in the absence of  $\text{CaCO}_3$ . It is highly probable, therefore, that some soils contain *Azotobacter* but are incapable of initiating the growth of an *Azotobacter* film in a mannite culture solution.

Practically all soils that failed to produce *Azotobacter* films formed more or less heavy films of fungi in the medium containing  $\text{CaCO}_3$ . As a rule, no such films were formed in the medium containing no  $\text{CaCO}_3$ . Whether or not these fungi are associated with the increased

nitrogen fixation under these conditions is not known. It is possible that the films of aerobic fungi were a factor in maintaining anaerobic conditions and thereby stimulated nitrogen fixation by anaerobic organisms. The fungi were usually slow to develop, indicating that their development depended upon some subsequent change, possibly the accumulation of nitrogen or of calcium salts of some organic acids. The number of samples that failed to develop fungi films were hardly sufficient to give a comparison of the quantity of nitrogen fixed in the presence and in the absence of a film. It is perhaps significant, however, that the average quantity of nitrogen fixed by the 7 samples which failed to grow films of fungi in the presence of  $\text{CaCO}_3$  was only 2.6 mgm., compared with 6.0 mgm. for the 76 samples producing a film. This would indicate that the fungus growth is in some way associated with the fixation of nitrogen either as a factor or as a result.

It is evident from the preceding data that practically all soils will bring about the fixation of appreciable quantities of nitrogen under the conditions of these experiments. A large percentage of the soils examined however, failed to initiate the growth of *Azotobacter*. There are, therefore, other organisms which are capable of fixing appreciable quantities of nitrogen. Such organisms seem to be quite widely distributed in nature.

#### CONCLUSIONS

(1) The quantity of nitrogen fixed in the presence of *Azotobacter* is greater than when it fails to develop.

(2) The number of soils capable of initiating the growth of *Azotobacter* under the experimental conditions here described is greater by 20 per cent if  $\text{CaCO}_3$  is added to the medium than if it is omitted.

(3) The quantity of nitrogen fixed in a medium containing  $\text{CaCO}_3$  is, for practical purposes, always equal to and in most cases greater than when  $\text{CaCO}_3$  is not present in the medium.

(4) The presence of  $\text{CaCO}_3$  exerts a greater beneficial effect upon those organisms, other than *Azotobacter*, that bring about the fixation of nitrogen than upon *Azotobacter* itself.

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## GUMMOSIS OF CITRUS<sup>1</sup>

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### PART I.—GUMMOSIS DUE TO *PYTHIACYSTIS CITROPHTHORA*

#### INTRODUCTION

The purpose of Part I is to present the results of an investigation, begun in 1912, into the nature, causes, and manner of development of certain types of gummosis of Citrus trees. In the three parts of this paper the term gummosis will be employed in the broader sense, in which it applies not only to the process of gum formation but also to the diseases or pathological effects in which gum formation is one of the conspicuous features. When employed in connection with specific diseases, supplementary terms will serve to show its modified meaning.

Part I will deal mainly with the causal relation of *Pythiacystis citrophthora* Sm. and Sm. to one of the most widespread and destructive forms of Citrus gummosis in California. The relation of another fungus, *Phytophthora terrestris* Sherb., to a similar disease, mal di gomma, in Florida is also included. Part II will present the results of an investigation into the relation of *Botrytis cinerea* Lk. and other fungi to other types of gummosis in Citrus. The last section of this paper, Part III, will deal with gum formation as such, the conditions influencing its formation, and its relation to diseases. The results of investigations on the control of some of these diseases will be discussed in a bulletin to be issued from the California Agricultural Experiment Station and will therefore receive only a brief mention in this paper. A preliminary report regarding control has already been published by Fawcett, (24,26).

Previous investigators had come to the conclusion that all gum diseases of Citrus trees in California originated independently of microorganisms, according to Smith and Butler (56). It was held that these diseases were largely autogenous in their nature and were frequently induced through the effects of certain climatic or soil conditions alone. It now appears evident that these environmental conditions can not by themselves initiate all the severe forms of gummosis in Citrus earlier attributed to them, although many of these factors are found to play, as they do in most parasitic diseases, an important rôle as contributing

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<sup>2</sup> This investigation was started in the spring of 1912 under the auspices of the State commission of horticulture and was transferred to the University of California in the fall of 1913. The author wishes to acknowledge the encouragement given by Dr. A. J. Cook and Prof. R. E. Smith at the beginning of the work, the assistance rendered by J. D. Culbertson, J. A. Prizer, and many other Citrus growers. Acknowledgment is also due to Bruce Douglas for laboratory assistance during a portion of the time of the investigation and to those members of the staff of the College of Agriculture who aided by many suggestions and criticisms.

conditions which favor infection and invasion of the host by causal parasites.

Since, however, a nonparasitic explanation for these gum diseases in California had become so firmly established in the literature of the subject, it has been necessary to carry on a large series of carefully controlled experiments before concluding that fungi are necessary factors in the initiation and development of certain of these diseases. For the same reason the author deems it necessary to present his data in considerable detail.

The failure to recognize the parasitic factor in previous investigations, as regards *Pythiacystis gummosis* at least, is probably due in part to the following facts which will be brought out in more detail later: (1) The fungus initiating the disease is found in the bark in its vegetative stage only; (2) it dies out rapidly in tissue already invaded, leaving only a fringe or band of live mycelium at the advancing edges of the invaded tissues; (3) under adverse temperature and moisture conditions the organism frequently dies completely in the tissue, especially on resistant varieties, giving the appearance of a pure physiological effect; (4) in advanced stages the stimulus to gum formation spreads out far in advance of the band of invading mycelium, producing an outer gummosis zone in which localized gum pockets are frequently formed. This outer gummosis zone is usually free from the causal organism. Any attempt to find the organism in regions other than the narrow band at the outer rim of the invaded zone would result in failure.

#### HISTORICAL REVIEW OF CITRUS GUMMOSIS

In this historical review of the literature of Citrus gummosis it has been found impossible to separate with certainty the different forms of gummosis which recent investigations have shown to be distinct. It is believed, however, that most of the more destructive, rapidly developing forms mentioned by various writers were similar to those represented by *Pythiacystis gummosis* in California and *mal di gomma* in Florida.

Two of the earliest European writers on Citrus, Ferrari (29, p. 156-158) in 1646<sup>3</sup> and Sterbeek (60, p. 177) in 1682, briefly discuss certain forms of Citrus gummosis occurring in the orange plantings of Europe. Two other early writers on Citrus referred to by Savastano (52) as mentioning some minor form of gummosis were Clarici in 1726 and Corrado in 1787.

The first highly destructive type of gummosis of Citrus on record appeared in the Azores in 1834. Sweet-orange trees which had grown to the age of 200 to 300 years, and which were producing 6,000 to 20,000 oranges apiece, were found by Fouque (32, p. 837) to be affected with a very destructive form of gummosis (1). Yellow gum is mentioned as exuding on the trunks near and sometimes beneath the surface of the ground. The trees put on heavy crops of fruit, and the leaves turned yellow and fell off in great quantities. This brief description appears to indicate a form of disease resembling *Pythiacystis gummosis*. It was supposedly transferred from the Azores to the vicinity of Lisbon, Portugal, where a similar disease appeared in 1865.

A similar type of Citrus gummosis, according to Savastano (52), was extremely destructive in many Mediterranean localities. It was established in Messina, Italy, in 1863 and was reported near Reggio in 1864.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," pp. 232-235.

The disease advanced rapidly from Messina, passing into the province of Catania to Acireale. In 1865 it was established at Palermo, raging severely there until 1870. In all the Sicilian Citrus orchards the gummosis was present in such intensity as to constitute a true epidemic which destroyed all the trees. The orchards were later replanted with sour-orange stocks. In 1870 the disease was established in the region of Genoa. It became scattered (not epidemic) in the orchards of the Naples, Amalfi, and Gargano regions. Briosi (11) estimates the damage from gummosis in Italy as \$2,000,000 from 1862 to 1878.

In Greece, in Tunis, and in Spain gummosis became distributed in varying degrees of intensity. Briosi refers to its destructiveness in the Balearic Islands (near Spain) in 1871. In more recent years a similar disease has been reported in the Oasis of Tripoli by Leone (44) in 1918. Gummosis was attracting attention in Cape Colony, South Africa, in 1891.

In Australia, a destructive gummosis is referred to by Alderton (3) as occurring in New South Wales between 1860 and 1870, by McAlpine (46) as occurring near Sydney in 1867 and in Queensland in 1876. It is reported by Kirk (43) in New Zealand in 1885.

In the United States, records place its appearance at about the year 1875 in California according to Mills (48) and 1876 in Florida by Curtiss (17). In Florida, as footrot or mal di gomma, it attracted serious attention in 1879, following a very wet year according to Hume (41). Moore (49, p. 128-132), in 1881, speaks of its recent appearance, and Swingle and Webber (64) in 1896 report it as still gradually spreading. In California, gummosis was a serious trouble in nearly every Citrus locality by 1878. It was spoken of by Garey (34, p. 81-82) as the only Citrus disease of importance at that time. The horticultural literature of this period indicates that the discontinuance of the use of the common lemon, lime, and citron as stocks and the general adoption of the sour orange and sweet orange as the principal stocks in California were due to this disease.

Some of the other localities in the American continents where gummosis has been an important disease are reported in Paraguay by Bertoni (9), in Brazil by Aversa-Sacca (5), in Mexico by Gandara (33), in Cuba by Cook (15) and Cook and Horne (16, p. 35), and in Porto Rico by Stevenson (62).

Viewing the history of Citrus gummosis from the investigational standpoint, Briosi (11) studied a gummosis (mal di gomma) in Italy and described a fungus, *Fusarium limoni* Briosi, which he considered to be a factor in the development of the disease. McAlpine (46) regarded a similar type of severe gummosis in Australia as undoubtedly of an infectious nature and referred to the same fungus as the causal agent. His description of the disease indicates that it is similar in character to the form which Pythiacystis gummosis takes on large orange trees in California. It is quite possible that the "slender wandering filaments" which he found penetrating the tissue may have been those of a Pythiacystis-like fungus. In this connection it is of interest to note that in a letter to the writer in February, 1917, G. P. Darnell-Smith of the Department of Agriculture, New South Wales, reports finding *Pythiacystis citrophthora* on specimens of Citrus affected with a gum disease sent from the Norfolk Islands, east of Australia. Later, F. Stoward, in a letter of October 1917, reports having isolated *P. citrophthora* from lemon fruits in Western Australia and having confirmed its pathogenicity by inoculation.



Comes (14) produced gumming in Italy by inoculations with a bacterium which he called *Bacterium gummis* Comes. *B. gummis* is also mentioned by Averna-Sacca (5) in connection with gummosis of Citrus in Brazil and by Gandara (33) in connection with gummosis in Mexico.

A number of other investigators, among whom were Swingle and Webber (64), considered the severe gum diseases as probably infectious and due to some organism invading the bark, but little work of an experimental nature with Citrus appears to have been done until recent years. Fawcett (22 and 23) showed that a gumming of branches of Citrus in Florida was due to the presence of a fungus similar to *Diplodia natalensis* Evans. The same fungus was found by Burger (28) to be the causal agent in a twig disease of the peach.

Not alone in Citrus but in a number of other plants, especially Prunus, definite diseases accompanied by large gum exudations have been shown in recent years to be due to specific organisms. Among these may be mentioned forms of gummosis on cherries reported by Aderhold and Ruhland (2), Griffin (37), and Barss (8); on apricot and other deciduous fruits reported by Barrett (6); and on plum reported by Higgins (39).

There have been other investigators who concluded that the severe gum diseases in Citrus were due, not to the invasion of organisms, but to certain stimuli operating upon the affected parts. Savastano (51) made a comparative study of gummosis in both Prunus and Citrus and concluded, because the histology was the same in both genera, that gummosis in Citrus arose largely from wounds or traumatism. This conclusion was in agreement with the views of many previous investigators as to gummosis in Prunus. Among these were Sorauer (58) in Germany, Prillieux (50) in France, and others. Savastano, in a number of papers in recent years on gummosis in Citrus, has modified this earlier view. He has distinguished clearly between mere gum formation as a general phenomenon and gummosis in connection with definite diseases. In one of these later publications (52) he accepts Comes's (14) conclusions as to the bacterial origin of the definite disease type and concludes that the aggravating conditions or causes influencing the occurrence of gummosis are lack of light, clayey, water-holding soils, level ground as compared to hillsides, excessive moisture about the roots, wounds from grafting or from digging about the roots and so forth. Most of these contributing conditions are those favorable for gummosis due to *Pythiacystis citrophthora* in California, or mal di gomma due to *Phytophthora terrestris* in Florida. In this connection it is of interest to note that R. E. Smith (57) found lemon fruits affected by typical brownrot like that due to *Pythiacystis citrophthora*, in a low-lying, poorly drained grove in Sicily, where footrot was very prevalent as mentioned by Fawcett (25).

Still other investigators have concluded that organisms are not at all involved in the initiation of gummosis but that certain conditions within or without the host are solely responsible for the diseases. Bertoni (9) in Paraguay appears to have considered Psorosis and a *Pythiacystis*-like form as phases of the same disease and concludes that poor condition of nourishment is the primary contributing cause. Later (10) he believed that shade was a corrective for gummosis. Grossenbacher (38) concluded that untimeliness of bark growth in connection with drought and low temperatures was related in some unknown way to gummosis of the mal di gomma type in Florida.

The previous views regarding Citrus gummosis in California, based in part on investigations on *Pythiacystis* gummosis, are fairly well indicated by the following quotation from Smith and Butler (56):

The lesions, ulcers, or affected areas produced, are not primarily the seat of the trouble. They represent rather the effect of what may be called a general constitutional derangement showing itself by external outbreaks or symptoms at whatever points may chance to be most susceptible. . . . . What may be called a primary weakness exists back of the visible symptoms, and in this weakness can be sought the fundamental cause and nature of the disease. It is, therefore, not necessary to identify a parasite, or strikingly evident climatic or soil conditions, to account for diseases of this class. . . . . It may be said here without extended discussion, that in no case have we been able to recognize or demonstrate the presence of any fungus, bacterium, or other parasitic organism as the cause of any form of citrus gum disease.

Some of the paramount conditions mentioned by Smith and Butler as contributory to the occurrence of gum disease are the accumulation of soil against the trunk, excessive moisture where poor drainage or careless irrigation exists, the heaping of manure against the trunk, low budding, lack of loose aerated soil and sweet orange stock on heavy soils. It is now known that these conditions are favorable for infection by the fungus and the development of the disease.<sup>4</sup>

Previous investigations and observations on Citrus gum diseases had led, therefore, to three general hypotheses: (1) That gum diseases were brought about by organisms capable of infecting and invading the tissue under certain contributing conditions; (2) that gum diseases were due to wounds or other external stimuli other than microorganisms acting immediately on the parts affected; (3) that gum diseases arose auto-genously being due to internal derangements of the host brought about by or without the influence of certain factors of the environment acting on the host as a whole.

#### PYTHIACYSTIS GUMMOSIS

*Pythiacystis* gummosis, with its associated rot of the fruit, is probably the most widespread and destructive of the Citrus gum diseases. The most striking features of the disease on the common lemon,<sup>5</sup> which is the most susceptible form, are copious exudations of gum and large dead patches of bark on the trunk, followed by yellowing and dropping of leaves. On old sweet-orange trees and other partially resistant forms, the dead patches are usually smaller and show a greater tendency to become self-limited.

In the earlier stages of the disease (Pl. 1, A; 3, A; 5, A) the extent to which the bark is invaded by the fungus can only be determined by lightly scraping the thin surface of bark in the vicinity of the exuding gum until the green color is seen. The margin between the invaded tissue and the sound bark then shows only as a difference in color, the normal green shading off gradually into a drab. The bark is not softened but remains firm. After a considerable time it becomes sunken and begins to crack longitudinally.

<sup>4</sup> In this connection it should be mentioned that Smith (55) was also among the first to accept the conclusions to which the first experimental work by Fawcett (24) led, although these were opposed to the views which he had formerly held.

<sup>5</sup> The names of species and varieties of Citrus will be used in accordance with Swingle (63), as follows: common lemon, *Citrus limonia* Osbeck; rough lemon, a horticultural variety of *C. limonia* Osbeck; sweet orange, *C. sinensis* Osbeck; sour orange, *C. grandis* Osbeck; citron, *C. medica* Linn.; trifoliate orange, *Poncirus trifoliata* Raf. The word lemon when used alone will refer to the common lemon and the word orange to the sweet orange.

On healthy, rapidly growing lemon trees the area of killed and darkened bark, elliptical to irregular in outline, is usually 15 to 30 cm. in vertical length and half that in width, when the gum first becomes apparent. By that time the fungus has been invading the tissue usually for a period of from two to four months. The removal of the bark at this time will show that the outer margin of the invaded zone is about coextensive with that seen on the surface (Pl. 3, B). Most often in young trees the death of the cambium and inner bark precedes slightly the death of the outer bark. The upward extension from the point of infection is usually many times its lateral and usually much greater than its downward extension.

In the cambium region surrounding an actively invaded area evidence of an influence extending from the margins of the dead bark will be found. There is simply a production of clear, watery gum which seems to originate in the region of the embryonic wood among the live cells without any apparent fermentation or decay. This region which is not yet darkened beyond the invaded portion, will be spoken of in this paper as the "outer gummy zone." It may in time extend considerable distances upward and downward and small distances laterally from the margin of the invaded zone. It has been traced for 60 and 90 cm. upward. The extent of this outer gummy zone varies especially with the age and rapidity of development of the disease lesion and the condition of the tree. In some cases it is much larger than the invaded zone and in others much smaller.

The inner surface of the bark in the invaded zone in a lesion of considerable size varies from mineral brown to burnt umber or fawn color<sup>6</sup> and the same discolorations will be found on the surface of the wood just at or beneath the cambium. (Pl. 3, A, B.) The discoloration does not extend far radially (usually only 2 to 5 mm.) into the woody layers. The cambium region in the outer gummy zone is chamois to yellow ochre in color, fading out gradually at the margins into the normal white color of the sound woody surface.

Frequently when the bark is irregular in contour, gum pockets will be formed, 2.5 to 5 cm. in longest axis, due to the rapid and unequal formation of gum. The gum accumulates near the cambium and by pressure separates the bark from the wood at certain places, forming definite pockets. The pressure is usually relieved by a break in the bark before the pockets become large. A few deeper gum pockets of considerable size have also been found, situated in the outer gummy zone, beneath layers of wood 3 to 6 mm. in thickness, showing accumulations of gum under pressure. The gum, which is watery and clear when first formed, hardens as it comes to the surface, apparently by loss of water, and finally becomes brittle. On the surface the hardened gum usually ranges from mahogany to chestnut in color.<sup>6</sup> The gum accumulates on the surface in long narrow ridges (Pl. 1, A, B; 4, A; 5, A, D; 7, A) or in oval masses, or runs down and collects in masses on the soil, depending upon the rapidity of formation and the dryness of the air. During periods of heavy dews and rains it gradually dissolves and disappears (Pl. 1, D; 5, E).

Only in rare cases where the surface of the bark is moist during the development of the disease is there any sign of fungus development to

<sup>6</sup> RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912

be detected without a microscope, and even then it is somewhat difficult to see because of the hyaline nature of the vegetative hyphae. The invading hyphae frequently die out rapidly behind the marginal fringe of advance, and quite often they die out completely over a part or all of this outer margin, so that progress of the disease is checked or entirely arrested. Such cases are often found in trees having some resistance, especially in orange and pomelo trees, or where the weather conditions subsequent to infection become unfavorable to the parasite.

In trees on which the disease has been present for a long time, the dead bark over the invaded portions dries, shrinks, and cracks. The larger cracks are mostly vertical, with smaller horizontal cracks (Pl. 1, D; 2, D, E; 5, E, F). A thin layer of the wood immediately under the invaded bark will usually be found to be infiltrated with hardened reddish brown gum which protects the under layers from rapid drying out and appears to protect the wood to a considerable extent against the entrance of wood-rotting fungi.

On old sweet-orange trees, the invaded areas are usually less extensive and more restricted laterally than on the common lemon. There is usually less gum than on the lemon. With the orange there is greater tendency than with the lemon for the invading fungus to die out—for the invaded area to become self-limited. Frequently the invaded areas on old sweet-orange trunks extend upward from the soil surface as narrow tongues of killed bark. On younger and frequently on older vigorous orange trees growing on heavy clay soils the disease may assume much the same characteristics as on the common lemon.

#### ISOLATION AND IDENTIFICATION OF *Pythiacystis citrophthora*

Culture tests were made from different parts of gummosis lesions, beginning at the center of a lesion and taking samples about 1 cm. apart upward, downward, and laterally from the center. A number of organisms were found, as would be expected, in the dead and discolored bark tissue. From recently killed and dying bark tissue at the advancing margin of the darker areas (invaded zone) a fungus was obtained which was afterwards found to be identical with that producing brownrot mentioned by Smith and others (57) of lemon fruits and which, even before its identity with *Pythiacystis citrophthora* was known, was shown to be capable of reproducing the same type of bark disease. Beyond the margin of the killed areas, however, all through the outer gummous zone the inner bark tissue was usually found to be entirely free from microorganisms, although internal gumming had set in 15 to 60 cm. above and somewhat less below the margin of the killed tissue.

*Pythiacystis citrophthora* was first isolated from the bark of diseased lemon trees in September, 1912, at Whittier, Calif., but its identity was not recognized at that time.

The method which the author finally found most useful for determining the fungus was to cut out with a sterile scalpel small bits of diseased tissue from the margin of the invaded portion and place them in tubes of corn meal agar. After two to four weeks' growth on this medium the characteristic sporangia were formed. By this method pure cultures were readily obtained, and when other organisms were present as contaminations *Pythiacystis citrophthora* could be identified by its characteristic sporangia. The *Pythiacystis* may readily be separated from contaminating fungi by transferring the mixed culture to a jar of water

containing lemon fruits. The *Pythiacystis* will enter the fruit and produce brownrot, leaving the associated organisms behind. It may then be isolated more easily as a pure culture from bits of the infected fruit.

After the discovery that *Pythiacystis citrophthora* was capable of inducing the disease it remained to be shown that this fungus was commonly present in connection with this type of gummosis. Accordingly, a survey was made of various Citrus localities in California. During the investigations (1912-1916) *P. citrophthora* was isolated 109 times from the diseased bark of 68 Citrus trees, representing about 30 different orchards in 10 counties of California, extending from San Diego on the south to Butte County on the north. It was also isolated from one locality in Arizona (Table I). In addition, the fungus was reisolated in 40 different cultures from 20 cases of gummosis produced by inoculation.

TABLE I.—Isolation of *Pythiacystis citrophthora* from gummosis lesions from natural infections

Variety.	Season.	Number of localities.	Number of trees	Number of positive cultures.
Lemon.....	Spring.....	2	14	21
	Summer.....	8	19	32
	Fall.....	6	20	25
Sweet orange.....	Spring.....	5	5	12
	Summer.....	4	4	9
	Fall.....	2	4	4
Pomelo.....	Winter.....	1	1	4
	Spring.....	1	1	2

These 109 cultures were obtained from 53 lemon trees, 13 sweet-orange trees, and 2 pomelo trees. All of the lemon, 9 of the sweet-orange, and the 2 pomelo trees were affected with typical gummosis of the trunks with the invaded portions of bark extending some distance above the surface of the soil. Four of the orange trees had lesions low down, near or upon the main roots, as is typical of mal di gomma or footrot lesions.

Although many isolation culture tests were made from various portions of the affected bark, including the outer gummous zone, and even some of the unaffected tissue, all of the successful isolation cultures except one were obtained from the advancing margin or outer fringe of the invaded zone. Isolation tests were made every month in the year except during January and February, and cultures of the fungus were obtained during each of the 10 months. The secondary organisms most commonly obtained in cultural tests from the older portions of the invaded zone back of the margins were species of *Fusarium*, *Alternaria*, *Cladosporium*, and *Colletotrichum*, and a large number of bacterial species.

#### INOCULATIONS WITH DISEASED TISSUE

Even before the causal fungus was discovered the infectious nature of the disease was determined by means of a large number of inoculations with diseased bark, the results of which are represented by the following typical examples.

On February 27, 1912, at Chula Vista in San Diego County, the following inoculations were made on 16-year-old lemon trees in heavy soil. The inocula were inserted into vertical cuts about 2 cm. long, made

through the bark of healthy tree trunks after first washing the surface with water and with alcohol. Paraffined paper was then tied over the cuts, control cuts without inoculum being used for comparison. In these preliminary experiments, the inocula used were as follows: (1) Bits of diseased bark tissue from near the margin of an actively enlarging gummosis lesion; (2) a bit of gum-filled woody tissue taken just outside the area of killed bark, in the outer gummous zone; (3) a piece of exuded gum from the same lesion; (4) bits of bark from a sound, healthy tree (used as controls). A cut was also made without inserting inoculum of any kind.

Of these inoculations, the first (consisting of two trees) brought about a development of the disease beginning at the point of inoculation. The other cuts all healed without any exudation of gum or visible injury to the bark. It will be noticed that the inoculations resulting in disease were made from tissue cut from the advancing edges or margins of recently killed tissue, while the others were from tissue or gum beyond this killed area (outer gummous zone) or from healthy bark. The development of the disease in one tree will be described, since it proved to be typical of the severe form of *Pythiacystis* gummosis as it occurs on lemon trees in the coastal sections of California.

On April 24, 1912, about two months after inoculation, gum was exuding rapidly and flowing downward to the surface of the soil, 15 cm. below, and hardening in a 15-mm. ridge. On August 2, over five months after inoculation, the exterior area of discolored bark was 15 by 25 cm. with a copious exudation of gum, forming three ridges on the bark surface and with a large mass of gum on the surface of the soil (Pl. 1, A). The foliage on the inoculated side of the tree was beginning to turn yellow. On September 19, 1912, the area of killed bark had extended upward and laterally, until it occupied a space 30 by 30 cm. and covered one-third the circumference of the trunk (Pl. 1, B). On November 15, 1912, the bark was dead on half the circumference of the trunk and the gum had formed ridges on the surface. The leaves on the affected side of the tree were very yellow and were dropping. The foliage on the side not affected was still healthy and normal. This tree now presented the appearance typical of many trees in the same locality which were affected naturally with *Pythiacystis* gummosis. On March 11, 1913, a little over a year from the time of inoculation, only 10 cm. of the 90-cm. circumference of the tree contained live bark at the level of the greatest lateral extension of the disease. At the place of greatest extension upward (46 cm. from the surface of the soil), the disease was arrested and callus tissue had formed. On May 24, 1913, the invaded area had extended only slightly beyond that of the previous observation. Pieces cut from the margins of the drying bark (Pl. 1, D) yielded cultures of *Pythiacystis citrophthora*. Most of the gum first formed had been dissolved and carried away by the rains. The bark on the side first affected had dried and contracted, showing longitudinal fissures just like those seen in old naturally occurring cases. Gum was exuding on the margins of the killed bark. In every respect this was a typical case of the form of gum disease due to *P. citrophthora*. On September 3, 1913 (18 months after inoculation) only about 5 cm. of live bark remained on the circumference of the trunk (Pl. 1, C), and the tree was considered useless from a commercial standpoint.

Many other series of inoculations were made with tissue from *Pythiacystis gummosis* lesions in different localities, varying widely in climatic and soil conditions, with the same general results as to type and progress of the disease. Inoculated trees contracted the disease just as readily on light sandy soils in orchards in which *Pythiacystis gummosis* had never occurred before as in orchards on heavy soils in which the disease had previously prevailed (Pl. 1, E, F). Variations in the development of the disease in individual trees and at various seasons of the year are indicated in Table II, which summarizes some of the inoculations with tissue from diseased lesions.

TABLE II.—Inoculations on trunks of 16-year-old lemon trees at Chula Vista, with tissue from diseased lesions<sup>1</sup>

Experiment No	Date of inoculation.	Date of observation.	Size of area <sup>2</sup>	Gum formation <sup>3</sup>
			Cm —	
1.....	Feb. 27, 1912	Mar. 24, 1912	—	3
		Aug. 2, 1912	25 by 15	3 (Pl. 1, A.)
		Sept. 19, 1912	30 by 30	3 (Pl. 1, B.)
		Nov. 15, 1912	33 by 30	1
		Mar. 11, 1913	48 by 80	—
		May 24, 1913	—	1 (Pl. 1, D.)
		Sept. 3, 1913	—	3 (Pl. 1, C.)
		Nov. 15, 1912	—	3 (Pl. 1, F.)
		Mar. 11, 1913	20 by 11	3
		May 24, 1913	38 by 11	3
2.....	Sept. 21, 1912	July 9, 1913	60 by 13	—
		Sept. 3, 1913	66 by 15	—
		Oct. 31, 1913	68 by 16.5	2
		Apr. 18, 1914	75 by 16.5	—
		Nov. 15, 1912	—	3 (Pl. 1, E.)
		May 24, 1913	40 by 22	2
		July 9, 1913	60 by 22	3
		Sept. 3, 1913	66 by 22	—
		Oct. 31, 1913	70 by 22	—
		Apr. 19, 1914	84 by 22	—
3.....	do.....	Feb. 6, 1913	—	3
		Mar. 11, 1913	13 by 8	—
		May 24, 1913	29 by 13	2
		July 9, 1913	46 by 33	3
		Feb. 6, 1913	—	0
		Mar. 11, 1913	7.5 by 5	1
		May 24, 1913	16.5 by 5	3
		July 9, 1913	16.5 by 5	—
		Sept. 3, 1913	21.5 by 7.5	— (Pl. 7, A.)
		Oct. 31, 1913	23 by 10	3
4.....	Nov. 16, 1912	Mar. 11, 1913	13 by 8	—
		May 24, 1913	29 by 13	2
		July 9, 1913	46 by 33	3
		Feb. 6, 1913	—	0
		Mar. 11, 1913	7.5 by 5	1
		May 24, 1913	16.5 by 5	3
		July 9, 1913	16.5 by 5	—
		Sept. 3, 1913	21.5 by 7.5	— (Pl. 7, A.)
		Oct. 31, 1913	23 by 10	3
		Oct. 31, 1913	23 by 10	3
5.....	do.....	Mar. 11, 1913	13 by 8	—
		May 24, 1913	29 by 13	2
		July 9, 1913	46 by 33	3
		Feb. 6, 1913	—	0
		Mar. 11, 1913	7.5 by 5	1
		May 24, 1913	16.5 by 5	3
		July 9, 1913	16.5 by 5	—
		Sept. 3, 1913	21.5 by 7.5	— (Pl. 7, A.)
		Oct. 31, 1913	23 by 10	3
		Oct. 31, 1913	23 by 10	3

<sup>1</sup> All experiments except No. 1 were made on trees growing in light sandy soil.

<sup>2</sup> The first number gives the greatest extension vertically and the second number the greatest extension horizontally. This applies also to similar data in the tables that follow.

<sup>3</sup> 0=none; 1=slight; 2=medium; 3=copious; a dash indicates that no data were obtained.

#### INOCULATIONS WITH DISEASED FRUITS

After it seemed probable that the fungus commonly associated with *Pythiacystis gummosis* was the same as that causing brownrot of lemon fruits, *Pythiacystis citrophthora*, it was of interest to determine whether the disease could be induced directly from the brownrotted fruits, either in wounds or on uninjured tissue.

Experiments were made (1) by inserting pieces of lemon fruits affected with the brownrot into incisions made in the bark of healthy lemon trees; (2) by placing similarly affected lemon fruits in contact with the roots or trunks of healthy lemon trees and covering them with moist soil; and (3) by placing affected lemons, as in experiment 2, at the root or trunk of orange trees budded on both the sour- and the sweet-orange stocks.

In a typical experiment of the first kind, a piece of diseased lemon rind was inserted into a cut in the bark of a 19-year-old lemon tree 20 cm. above the sweet-orange stock, on February 26, 1913. A control cut not inoculated healed rapidly without gumming, but in 45 days the inoculated cut showed definite evidence of infection. The killed bark area enlarged from 4 by 7.5 cm. in 70 days to 28 by 82 cm. in about 13 months after inoculation (Pl. 2, A, B, C). In about 18 months the killed area reached extreme dimensions of 35 by 125 cm., one-third of this length being upon the sweet-orange stock (Pl. 2, D); the leaves were now yellow and dropping on the affected side. Cultures were made from the bark and *Pythiacystis* was obtained, but only at the advancing discolored margins of the area where the bark was killed through to the wood. Specimens taken from other places in the diseased tissue, and from the live tissue just outside the diseased areas, either yielded other organisms or were sterile. Among the organisms found both inside and outside the area of recent invasion were species of *Colletotrichum*, *Alternaria*, and *Cladosporium*, and also species of bacteria.

The disease having been produced by inoculation from diseased fruits into cuts in the bark, it now became of interest to determine whether infection could originate from diseased fruit placed near uninjured bark.

On May 13, 1913, at Whittier, several lemon fruits affected with *Pythiacystis*-rot were buried in the soil near the trunk of a small lemon tree growing in a large earthen pot. The soil was heaped up against the trunk and kept moist by frequent watering. A similar tree in another pot received the same cultural treatment without the diseased fruit. During the summer of 1913 no results of infection were noted. When next examined, in March, 1914, the first tree was seen to be badly affected with typical *Pythiacystis* gummosis, while the control tree remained healthy.

In other tests on lemon and sweet-orange trees with decayed fruits, infection leading to *Pythiacystis* gummosis frequently but not invariably resulted. Sour-orange trees under the same conditions whether injured or uninjured failed to contract the disease.

These tests, taken in connection with numerous observations in orchards, show that the sour-orange tree is highly resistant to *Pythiacystis* gummosis and that sweet-orange and common-lemon trees, though easily infected through injuries, are not otherwise readily infected except under the most favorable conditions for invasion by the parasite.

#### INOCULATION WITH PURE CULTURES OF *PYTHIACYSTIS CITROPHTHORA*, AND REISOLATION

As a result of a large number of inoculations from pure cultures of *Pythiacystis citrophthora*, it was shown that this fungus was capable of invading the bark and bringing about all the characteristic effects that have been noted in naturally occurring cases or those produced by



inoculations with diseased bark or fruit. The following is a typical example.

On November 23, 1912, a lemon tree about 18 years old, having a trunk about 30 cm. in diameter, growing at Santa Paula, was inoculated by inserting a very small bit of mycelium from a culture of *Pythiacystis citrophthora* into a 2-cm. vertical cut made 15 cm. above the bud union. The surface of the bark had previously been washed with water and with alcohol and the inoculated cut was covered with oiled paper.

No effect from the inoculation was evident in 37 days, but in 42 days an invaded surface area of 5 by 9 cm. was observed, and this increased to 8 by 11 cm. in 76 days and to 8 by 35 cm. in 7 months, with copious exudation of gum (Pl. 3, A). A similar cut without inoculum, on the opposite side of the same tree, healed rapidly.

A strip of bark 10 by 68 cm., as seen in Plate 3, B, was cut out at this time, for the purpose of cultural examination and for enzym experiments, to be discussed in Part III of this paper. Cultures made from this strip of bark at various places, by cutting bits of tissue from the inner side, yielded *Pythiacystis citrophthora* at both the upper and the lower margin of the invaded zone (see white line shown on the plates) but not at points 25, 8, and 16 cm., respectively, above this killed area, within the outer gummous zone.

On removal of the bark, the cambium adjacent to the dark brown or blackened area appeared yellowish. This discoloration extended only slightly laterally but to a much greater distance from the margins of the cut upward and downward. In this outer gummous zone interior gum had formed in places 60 cm. or more upward from the killed and invaded margins. The causal organism, as indicated by isolation tests in numerous other cases, does not extend into this outer gummous zone. To remove the causal organism in treatment, therefore, it is necessary to cut away the bark only a short distance beyond the discolored region. Experiments have shown that when this is done the gum will cease forming, the further extension of the yellow, gummy zone will stop, and the bark over it will usually return to normal condition.

The cut-out area and surrounding bark shown in Plate 3, B, was painted with Bordeaux paste. Three weeks later, June 27, 1913, gum was seen to have continued to ooze out at the upper angle, but no further bark had died. The same tree is shown in Plate 3, C, in September, 1914, and in Plate 3, D, in June, 1920. No effect on the foliage or health of the tree, such as is usually seen in severe cases of *Pythiacystis* gummosis, was noticed in this tree. The organism was probably removed in time to obviate visible injurious effects.

The results of a number of other inoculations on lemon trees with cultures of *Pythiacystis citrophthora* are summarized in Table III; others on lemon, orange, and other Citrus varieties, will be found described under "Resistance" and "Mal di gomma." In all, about 90 inoculations with pure cultures resulted in *Pythiacystis* gummosis, from 20 of which the organism was reisolated.

TABLE III.—Inoculations of *Pythiacystis citrophthora* on trunks of 19-year-old lemon trees at Santa Paula

Experiment No.	Part of trunk inoculated.	Date of inoculation.	Date of observation.	Size of killed bark lesions.	Gum formation. <sup>1</sup>
1	30 cm. from bud union, 60 cm. from soil.	Nov. 23, 1912	Dec. 30, 1912 Feb. 12, 1913 Mar. 14, 1913 Apr. 12, 1913	0 10 by 3.5 11.5 by 5 18 by 8	0 3 3 3
2	15 cm. above bud union.	.....do.....	Dec. 30, 1912 Feb. 12, 1913 Mar. 4, 1913 Apr. 12, 1913 May 7, 1913 June 5, 1913	0 9 by 5 9 by 5 13 by 8 13 by 8 35 by 8	0 3 3 3 3 2 (Pl. 2, F, 3, A)
3	10 cm. above bud union.	.....do.....	Dec. 30, 1912 Feb. 12, 1913 Mar. 14, 1913 Apr. 12, 1913 May 7, 1913 June 5, 1913	0 14 by 5 18 by 5 19 by 6.5 19 by 6.5 25 by 6.5	0 1 3 3 — 3
Ar 74	Trunk, 13 cm. above bud union.	Feb. 20, 1913	Apr. 1, 1913 May 7, 1913 June 27, 1913 July 28, 1913 Oct 23, 1913	..... 7.5 by 4.0 10 by 6 15 by 8 33 by 15	2 3 3 3 3

<sup>1</sup> 0=none, 1=slight, 2=medium, 3=copious.

These typical examples show that the general development of the disease was the same with inoculations with pure cultures of *Pythiacystis citrophthora* as it was with the inoculation with bits of diseased bark or fruit previously described. A period of slow development for 2 to 4 months is followed by copious gumming and rapid development, resulting in large invaded areas in 6 to 10 months. Later the rate of increase is again comparatively slow and is frequently partially checked. It is believed that the accumulation of gum in the outer gummy zone is a material hindrance to the rapid advance of the fungus. This feature of the disease is discussed in Part III of this paper.

A partial representative list of inoculations from which *Pythiacystis citrophthora* was again isolated, with the date and the size of invaded areas at time of isolation, is shown in Table IV.

TABLE IV.—Representative inoculations of *Pythiacystis citrophthora*

Date of inoculation.	Date of re-isolation.	Size of invaded area.
Nov. 16, 1912.....	Nov. 1, 1913.....	Cm.
Do.....	July 10, 1913.....	23 by 10
Nov. 23, 1912.....	Apr. 12, 1913.....	46 by 15
Do.....	June 5, 1913.....	47 by 8
Do.....	do.....	35 by 8
Feb. 26, 1913.....	Feb. 7, 1915.....	25 by 6
Do.....	Oct. 22, 1913.....	95 by 46
July 13, 1913.....	May 9, 1914.....	33 by 13
Sept. 10, 1914.....	Oct. 9, 1914.....	48 by 15
Oct. 14, 1914.....	Apr. 7, 1915.....	15 by 5
Feb. 7, 1915.....	Sept. 3, 1915.....	25 by 8
Mar. 4, 1915.....	do.....	8 by 5
Do.....	do.....	30 by 10
Apr. 9, 1915.....	do.....	46 by 12
		30 by

As is shown by Table IV, the fungus was recovered in different cases from 1 to nearly 12 months after inoculation and from margins of different invaded areas varying from 8 to 95 cm. in vertical extent. Since the fungus was isolated most frequently at the upper margin of the invaded area, the distances from the original point of inoculation at which the fungus was recovered was frequently more than one-half that of this vertical extension, because the areas usually enlarged upward much faster than downward or laterally, as previously pointed out.

The severe inoculation test to which this fungus was subjected in order to establish its causal relation to this type of gummosis is indicated by an example of the repeated isolations, inoculations and reisolations of one of the strains of this fungus, over a period of three years. Isolated in September, 1912, its record was as follows:

Date of inoculation.	Date of reisolation.	Size of lesions.
Nov. 23, 1912.....	Apr. 12, 1913.....	Cm. 47 by 8
June 13, 1913.....	May 9, 1914.....	48 by 15
Mar. 4, 1915.....	Sept. 3, 1915.....	46 by 13

The fungus was thus in the bark of the three trees for periods of about 5, 11, and 6 months, respectively, and between these periods in cultures for about 2, 2, and 10 months, respectively. Transfers from the original culture which were kept alive for over 8 years, on cornmeal-agar medium, were still capable of producing brownrot of lemon fruits when tested in 1921.

#### INOCULATIONS WITH *Pythiacystis citrophthora* ON DIFFERENT PARTS OF SAME TREES

After the results of the first inoculations began to indicate that *Pythiacystis citrophthora* was the causal agent in this type of disease, it became important to find out the effect of the fungus when introduced into different parts of the same tree. Inoculations were made on roots, trunk, and branches of various ages, as shown in Table V.

TABLE V.—Inoculations with *Pythiacystis citrophthora* July 13, 1913, at different locations on same tree

Part of tree inoculated	Length and width of killed bark areas.					Final extreme distance from place inoculated.	
	1913			1914			
	July 27.	Sept 13.	Nov. 25.	Apr. 9.	May 9.	Upward.	Downward.
Large root (sweet-orange stock), 13 cm. below bud union (Pl. 4, B) . . . .	Cm. 2.5 by 1.8	Cm.	Cm.	Cm. 13 by 4	Cm. 14 by 5	Cm. 9	Cm. 5
Orange bark of stock 5 cm. below bud union (Pl. 3, D) . . . . .	4 by 1.8	10 by 2.5	24 by 10	43 by 13	48 by 15	40	8
Lemon trunk, 13 cm. above bud union (Pl. 4, B) . . . . .	5 by 2.5	25 by 10	50 by 25	53 by 25	68 by 25	46	22
Lemon trunk 88 cm. above bud union just below crotch . . . . .	2.5 by 0.6	13 by 4	34 by 9	36 by 9	36 by 9	.....	.....
Limb 8 cm. in diameter . . . . .	2.5 by 0.6	20 by 6	33 by 8	33 by 8	33 by 8	20	13
Limb 5 cm. in diameter . . . . .	.....	.....	30 by 5	30 by 5	30 by 5	18	12
Limb 5 cm. in diameter . . . . .	.....	.....	27 by 1.3	30 by 2.5	30 by 2.5	20	10
Limb 2.5 cm. in diameter . . . . .	.....	10 by 5	10 by 5	15 by 5	15 by 5	10	5
Limb 3 mm. in diameter . . . . .	.....	15 by 5	18 by 5	22 by 5	22 by 5	15	7
Controls, not inoculated . . . . .	0	0	0	0	0	0	0

The control cuts not inoculated healed without gumming or dying of bark. All cuts inoculated with *Pythiacystis citrophthora* resulted in killing of the bark accompanied by exudation of gum. The areas on the branches had nearly reached their maximum size when examined on November 25, 1913, 104 days after inoculation. A slight increase in some of them took place previous to the next April, at which time they had ceased to enlarge and had become self-limited, as was shown by subsequent records in Table V. The fungus apparently died out of these limb lesions, as no evidence of it could be found later.

The inoculation on the orange root resulted in gumming and killing of some bark in two weeks and continued gumming for about four months. This lesion (Pl. 4, C, lower area) became self-limited after an area 14 by 5 cm. had been killed and later resembled self-limited typical footrot or mal di gomma lesions as they often occur naturally on orange trees in Florida.

The diseased area resulting from inoculation on the orange bark 5 cm. below the bud union enlarged at about the same rate as that on the root for the first two weeks. After it had spread to the lemon bark, however, at Amud union, the progress upward was rapid. By May 9, 1914, the killed area was 48 by 15 cm. (Pl. 4, A, B), only 8 cm. of this extension being downward on the orange bark. Of five cultures taken about 10 months after inoculation from the margin of the invaded area (see chalk line on Pl. 4, A), three developed *Pythiacystis citrophthora*. Further extension of the area was prevented by cutting out the bark (Pl. 4, B) and painting the trunk with Bordeaux paste.

The result of the inoculation made on the lemon bark on the opposite side of the same tree a few centimeters above the bud union is shown in Plate 4, C, the killed area being at this time (April 9, 1914) 53 by 25 cm. with large quantities of gum exuding on the surface.

#### COMBINED EFFECTS OF PYTHIACYSTIS CITROPHTHORA AND FUSARIUM SP.

During the examination of a large number of naturally occurring cases of *Pythiacystis* gummosis it was noticed that a species of *Fusarium* frequently accompanied and was closely associated with *Pythiacystis citrophthora* in the diseased tissue. The question arose as to whether the *Fusarium* played any part in the development or the severity of the disease.

*Fusarium* has been mentioned frequently in literature as having some possible relation to certain types of gum disease. Briosi (11) and McAlpine (46) concluded that *Fusarium limoni* Briosi played an important part in mal di gomma in Italy and Australia. Earle and Rogers (21), though not able to produce gummosis by inoculation with *Fusarium*, believed that under certain conditions it was probably a factor in a certain type of gum disease in Cuba. The writer had previously also found species of *Fusarium* repeatedly associated with mal di gomma or footrot in Florida, but inoculations with them had been negative.

Cuts, as before, into which bits of pure cultures of *Pythiacystis citrophthora* and *Fusarium* sp. were placed side by side were now made on lemon trees. At the same time inoculations into other similar trees were made with *P. citrophthora* alone and others with *Fusarium* sp. alone. The main results are given in Tables VI and VII.

TABLE VI.—Inoculation tests with *Pythiacystis citrophthora* alone, compared with those with *P. citrophthora* plus *Fusarium* sp.

INOCULATIONS MADE LOW ON TRUNKS OF 19-YEAR-OLD LEMON TREES, FEBRUARY 20, 1913

Date of observation.	<i>P. citrophthora</i> alone.		<i>P. citrophthora</i> and <i>Fusarium</i> sp.	
	Size of lesions.	Gum formation. <sup>1</sup>	Size of lesions.	Gum formation. <sup>1</sup>
1913.	Cm.		Cm.	
Apr. 1.	0.....	1	0.....	1
May 7.	7.5 by 3.8.....	3	6.3 by 2.5.....	3
June 27.	10 by 6.3.....	3	13 by 4.....	3
July 28.	15 by 7.5.....	3	30 by 9.....	3
Oct. 23.	33 by 13 (treated).	3	No record.....	
Nov. 25.			58 by 18.....	3

INOCULATIONS MADE HIGH ON TRUNKS OF 21-YEAR-OLD LEMON TREES, FEBRUARY 20, 1915

1915.	Cm.		Cm.	
Mar. 4.	0.....	0	0.....	0
Apr. 21.	2.5 by 2.5.....	1	5 by 2.5.....	3
May 18.	6.5 by 2.5.....	2	9 by 7.....	3
Sept. 4.	8 by 5.....	2	38 by 15.....	3

<sup>1</sup> 0=none; 1=slight; 2=medium; 3=copious.TABLE VII.—Inoculation with *Fusarium* sp. at Santa Paula.

Experiment No.	Date of inoculation.	Maximum results as to—		
		Gum. <sup>1</sup>	Initially killed bark at inoculation point. <sup>1</sup>	Cracking of outer bark. <sup>1</sup>
1	Nov. 23, 1912.....	2	1	1
2	do.....	1	0	1
3	Feb. 20, 1913.....	0	1	0
4	do.....	0	1	2
5	Mar. 14, 1913.....	1	1	1
6	do.....	0	1	1
7	do.....	1	1	1
8	Feb. 25, 1915.....	0	1	0
Control cuts without inoculum, to correspond with each of the above.....		0	0	0

<sup>1</sup> 0=none; 1=slight; 2=medium.

The progress of the disease on the tree inoculated with *Pythiacystis* and *Fusarium* on February 20, 1913, is more completely shown by Plate 5. The invaded area which was 58 by 18 cm. on November 25, 1913 (Pl. 5, B), the last date given in Table VI, had increased to 90 by 41 cm. on February 6, 1915 (Pl. 5, C-F). Extension vertically remained at 90 cm., but the lateral extension had increased to 61 cm. by March 9, 1916; and subsequently the entire circumference was encircled and the tree killed by the disease.

In the comparative inoculations with *Fusarium* alone on February 20, 1913, and February 25, 1915, No. 3 and 8 (Table VII), only a narrow layer of tissue along the cuts was killed, without exudation of gum. A thin outer layer of bark subsequently died about one inoculation, but otherwise the effect was not different in either case from that produced by the uninoculated cuts on the same trees. In a number of other inoculations with species of *Fusarium* associated with gummosis shown in Table VII, only slight effects in killed tissue and only slight to medium effects in gum formation were obtained.

Although the experiments on this phase of the question have been too few as yet to justify definite conclusions, the results suggest that the severity of the disease may be slightly increased by adding *Fusarium* sp. to *Pythiacystis citrophthora* at the time of inoculation. The characteristics of the disease except in rapidity of development, however, were the same as when *P. citrophthora* was inserted alone.

#### RESISTANCE OF DIFFERENT SPECIES AND VARIETIES OF CITRUS TO PYTHIACYSTIS GUMMOSIS

Among the Citrus species and varieties that have been tested, the common lemon has the lowest resistance to *Pythiacystis* gummosis and the sour orange the highest. The sour orange usually is so resistant to *Pythiacystis* attack that even when the most favorable conditions are given by inoculation in wounds there is only a slight gumming with rapid healing of the wounded tissue and with total failure to produce a diseased lesion. The sour orange is also highly resistant to all other infectious gum diseases of importance. Mere gum formation, however, may be induced by suitable stimuli in sour orange as well as in other species and varieties. Of the forms which have been most used for stocks, the trifoliate orange probably stands next to the sour orange in resistance and the sweet orange next to the common lemon in susceptibility, with the pomelo and the rough lemon between these two. Because these stocks are grown from seed there is a large possibility of variation in resistance within each variety, due to differences between "strains," and observations have indicated that such variation actually exists. The following observations and experiments indicate in a rough way the relative resistance of some of the species and varieties.

A block of 5,000 sweet-orange seedlings about 2½ years old, growing in nursery rows on medium heavy clay loam soil, had been planted adjacent to a block of 15,000 sour-orange seedlings of the same age and having the same care. All the trees had been irrigated rather frequently and heavily. On October 21, 1914, four representative rows of sweet-orange trees showed the following percentage of *Pythiacystis* gummosis:

Row No.	Number of trees in row.	Number of affected trees.	Percentage affected.
1.....	222	52	23
2.....	213	73	34
3.....	212	63	29
4.....	180	53	29
Total.....	827	241	.....
Average.....	.....	.....	29

On some trees only a small lesion was evident with much gum exuding, on others the bark was killed to a distance of 15 to 30 cm. above the soil, with an abundance of gum, and still other trees were dead. Some trees showed a strong tendency to form ridges of callous tissue along the edges of the dead strips of bark. A thorough search in the block of sour-orange trees failed to reveal a single tree affected.

Differences in resistance are indicated further by an estimate made by W. M. Mertz in a nursery of Citrus seedlings about 2 years old grown at the Citrus Experiment Station. The following is the percentage of gum disease (probably *Pythiacystis gummosis*) which was recorded:

Species.	Number of trees.	Percentage with gummosis.
<i>Citrus aurantium</i> (sour orange) .. . . . . .	1, 000	0. 3
<i>Poncirus trifoliata</i> (trifoliate orange) .. . . . . .	1, 000	1. 0
<i>Citrus grandis</i> (pomelo) . . . . .	1, 000	2. 5
<i>Citrus sinensis</i> (sweet orange) . . . . .	2, 000	10. 0

The inoculations recorded in Table VIII were made on trees about 2 years old from seed, by cutting through the bark on the stem not far from the soil and inserting bits of the mycelium from cultures of *Pythiacystis citrophthora* grown on sterilized orange wood. Oiled paper was tied over the cuts.

TABLE VIII.—Inoculations made May 15, 1916; observations July 2, 1915, when the plants were pulled up

Host.	Number of plants.	Results
Sour orange.....	3	No gum on exterior. One shows interior gum. All healing normally.
Rough lemon.....	5	No gum. Healing normally.
Sweet orange.....	4	Two trees gumming copiously; long strip of bark killed. Two trees no gum on surface; wounds healing.
Pomelo.....	3	Bark killed slightly on edges of cuts without gum on exterior, healing rapidly.

Of the four forms tested in this experiment, sweet orange showed the greatest effect, pomelo less, and sour orange and rough lemon<sup>7</sup> showed no appreciable effect from the inoculation. The weather was very hot and dry during the experiment and thus not very favorable to the fungus.

On February 6, 1915, inoculations into the trunks of 21-month-old seedling sour-orange and rough-lemon trees were made at Santa Paula. A branch of a young common-lemon tree close by was inoculated at the same time. The results on May 18, 1915, were as follows:

The inoculated sour-orange cuts healed rapidly, with slightly more gaping of the wound than in the controls, while the rough lemons showed a small amount of dead bark next to the inoculated cuts, which were also healing rapidly. A slightly lower resistance of the rough lemon than of the sour orange was indicated. On the common lemon, however, the leaves had withered on the inoculated branch, the bark was killed

<sup>7</sup> The rough lemon is thought to be a hybrid. It is used largely for stocks in Florida and should not be confused with the common lemon.

around the branch for a distance of 8 cm. below the wound, and a large quantity of gum had formed. The control cuts on all three varieties healed perfectly without gumming or evident injury to the trees.

On July 2, 1913, inoculations were made at Whittier, with *Pythiacystis citrophthora* in cuts on the bark of sour-orange, sweet-orange, and a number of young deciduous fruit trees. The results on September 26, 1913, are given in Table IX.

TABLE IX.—Comparative inoculations with *Pythiacystis citrophthora* into Citrus and deciduous fruit trees. Inoculations made July 2, 1913; observations, September 26, 1913

Inoculation No.	Host	Results.
1	Sour-orange stock (Valencia orange scion above).	Slight gumming, but healing rapidly.
2	Sour-orange stock (Valencia above)	Healed perfectly, no gumming.
3	Sour-orange stock (lemon above) . . .	Healed with swollen scar only.
4	Valencia orange scion, hardened tissue (sour-orange stock below).	Gumming copiously with killing of small amount of tissue.
5	Valencia orange, same tree as No. 4, younger tissue.	Gumming copiously; 15-cm. strip of bark on one side of twig killed.
6	Control cuts on sour-orange and on Valencia orange bark (not inoculated).	All healed perfectly without visible gumming on surface.
7	Almond (2 trees) . . . . .	Gum exuded. Tissue slightly killed beyond cut.
8	Almond (control, not inoculated) . . .	Healed perfectly without gumming.
9	Peach (12-mm. stem) . . . . .	Much exuded gum. Bark killed 5 by 1.3 cm.; killed wood 8 cm. long.
10	Peach (control, not inoculated) . . .	Healed perfectly with slight gumming
11	Burbank plum . . . . .	Gumming, outer part of bark killed and sunken, but healing underneath.
12	Burbank plum (control not inoculated).	Healed perfectly, no gum.
13	Pear . . . . .	Healed with enlarged scar.
14	Pear (control) . . . . .	Healed with slight scar.

As before, the sour-orange bark showed high resistance while the sweet-orange (Valencia) bark was considerably affected by the fungus. Of the deciduous fruits tested, peach was most affected, almond and plum slightly, and pear scarcely at all.

In Table X are shown the results of inoculation into branches of various species by placing bits of *Pythiacystis*-infected fruit into cuts.

TABLE X.—Comparative inoculations on Citrus branches, October 9, 1914; observations October 22, 1914

Experiment No.	Host.	Size of diseased lesions	Gum <sup>1</sup>
		Cm.	
1	Common lemon . . . . .	5 by 1.3	3
2	do . . . . .	5 by 1.3	3
3	Citron . . . . .	2.5 by 0.5	3
4	do . . . . .	2.5 by 0.3	3
5	Sweet orange (Valencia) . . . . .	4 by 1.0	3
6	Sweet orange (navel) . . . . .	3 by 1.0	3
7	Sour orange . . . . .	1.2 by 0.5	2
8	Controls . . . . .	0	0

<sup>1</sup> 0=none; 1=slight; 2=medium; 3=copious.



\* The lesions here, though small at the time of observation, give some indication of differences in resistance. The common lemon was most affected, followed by the sweet orange (Valencia and navel) and the citron. Sour orange, as before, was quite resistant, though showing some effect, but was already beginning to form callus at the cut.

Experiments to test the effect of *Pythiacystis citrophthora* on small roots of different species of seedlings were also made.<sup>8</sup>

On November 15, 1915, mycelium of *Pythiacystis* and lemon fruits affected with *Pythiacystis* in different tests were placed on the healthy roots (with and without punctures) of the common lemon, sweet orange, pomelo, and sour orange. When last examined, on May 11, 1916, only the roots of the common lemon, where inoculated with *Pythiacystis citrophthora* (with punctures) and where inoculated with diseased lemon fruit (without punctures), were killed. The fungus was reisolated from the lemon root which had been inoculated with *P. citrophthora*. This experiment gives additional weight to the correctness of previous observations that even small and medium sized roots of the common lemon are frequently attacked by *P. citrophthora*, but that the small roots of other varieties are quite resistant.

Inoculations reported in other sections of this paper also give further indication of differences in resistance, especially as between the common lemon and sweet orange. The reasons for these differences in susceptibility is an interesting question in itself, which has not as yet been investigated. Our inoculation experiments would indicate that the differences in resistance in sour orange, sweet orange, and lemon, at least, can not be confined to the superficial layers of cells. If it were so limited the insertion of the parasite into cuts should cause equal effects in each variety.

#### MAL DI GOMMA IN RELATION TO PYTHIACYSTIS GUMMOSIS

Mal di gomma, due to *Phytophthora terrestris* Sherbakoff, is a gum disease with close relationships to *Pythiacystis* gummosis. It has previously been pointed out that certain phases of the *Pythiacystis* gummosis occurring on or near the main roots of sweet-orange trees are similar to those of mal di gomma, or footrot.

For this reason, certain footrot-like forms due to *Pythiacystis citrophthora* in California have previously been known by the name of mal di gomma according to Smith and Butler (56), 1908, and Fawcett (26). Since the name mal di gomma was first used in Florida to designate a common Florida gum disease, which is now known to be due to *Phytophthora terrestris*, it is proposed to restrict its use (in this country at least) to the disease due to this fungus.

This type of gum disease affects, for the most part, the bark on the lowest portion of the trunk and the upper portion of the first main roots, mostly below the surface of the soil. Gum usually forms on the trunk of the tree above the soil. The inner bark and finally the wood under-

<sup>8</sup> Boxes were constructed with one side consisting of glass plates inserted into grooves and covered by a removable wooden door fastened by hooks to hold it against the glass. After the young trees had become established in these boxes, the glass plate was removed to make inoculations on roots that had grown out against it and was then replaced in its former position. The assistance of Mr. E. E. Thomas, for whom similar experiments with *Fusarium* were made, is here acknowledged.

neath frequently develop a disagreeable fetid odor.<sup>9</sup> The bark dies and breaks away in patches, leaving bare, dead areas, which spread in all directions, but mostly downward, on the main crown roots and laterally around the trunk. Trees thus affected bear heavy crops of fruit and the leaves become yellow.

*Phytophthora terrestris* Sherbakoff (53) was first isolated by the writer from gumming lesions of an orange tree at Lindsay, Calif., in 1912, and was considered at that time to be only a peculiar strain of *Pythiacystis citrophthora*. Later, in 1914, it was isolated from mal di gomma lesions, as follows: (1) from a grapefruit tree at Palmetto, Fla. (26, fig. 5); (2) from orange trees in Cuba; and (3) from a tangelo tree on the Isle of Pines. The Florida and Cuban cultures were still considered to be strains of *Pythiacystis citrophthora* and were referred to as such in publications (25, 26). Stevens (61) later made an extended survey and isolated this species in a large number of mal di gomma cases from widely separated localities representing the principal part of the Florida Citrus belt. In the meantime, Sherbakoff (53) had described *Phytophthora terrestris* as causing rot of tomatoes in Florida. The cultures previously isolated at Lindsay, Calif., and in Florida and Cuba were then examined by Sherbakoff, who concluded that they were all the same species. A culture isolated by George Fawcett from a lemon tree in Argentina and sent to the writer in October, 1916, was also determined to be similar to this species. Later, the writer (27) determined that *Phytophthora terrestris* had markedly different growth-temperature relations from those of *Pythiacystis citrophthora*. The vegetative growth on certain culture media, the method of forming sporangia, and the effect on the host when inoculated, are so similar, however, as to make it seem probable that the two are closely related species. (See inoculation experiments, Tables XI and XII.)

*Phytophthora terrestris* is believed by some authors to be identical with *P. parasitica* Dastur. Ashby (4) has recently described a leaf-stalk rot of coconuts in Jamaica as being due to this latter fungus and states that Dastur has compared *P. terrestris* with *P. parasitica* and found them to be identical. Ashby has also found this fungus on tobacco and pineapple plants. Dastur (19) originally described his species as causing a disease of castor oil plants in India and later (20) found it attacking *Vinca rosea*. If *P. parasitica* and *P. terrestris* are the same species, a wide distribution of this fungus is indicated.

#### Comparative Inoculations with *Phytophthora Terrestris* and *Pythiacystis*

Many different series of inoculation experiments with *Pythiacystis citrophthora* and *Phytophthora terrestris* were made under varying conditions, the results of some of which are given in Tables XI and XII.

<sup>9</sup> This rotting of the wood, as well as the bark, and the accompanying "fetid odor" are believed to be due mainly to secondary organisms setting up fermentation and decay below the surface of the soil after killing of the bark by the primary organism. While gum may be formed below as well as above the surface of the soil, it is dissolved readily by moisture and is usually less conspicuous below the soil surface.

TABLE XI.—Comparative effects of various "strains" of *Pythiacystis citrophthora* and *Phytophthora terrestris* on lemon and orange bark—Eureka lemon trunks about 6.5 cm. in diameter, budded high on sweet-orange stocks, at Riverside, Calif. Inoculations April 17, 1913; examination May 20, 1913

Experiment No.	Source of culture.	Host and place on tree inoculation.	Killed area.	Gum formation. <sup>1</sup>
			Cm.	
1	<i>Pythiacystis citrophthora</i> , from lemon bark, Whittier, Calif.	Eureka lemon above bud union.	3 by 1.3...	2
2	....do.....	Orange stock below bud union, same tree.	2 by 1.....	2
3	<i>Pythiacystis citrophthora</i> from lemon bark, San Diego, Calif.	Eureka lemon above bud union.	2.5 by 1.5..	2
4	....do.....	Orange below bud union..	0.....	0
5	<i>Phytophthora terrestris</i> from orange bark, Lindsay, Calif.	Eureka lemon above bud union.	4 by 2.5....	3
6	<i>Phytophthora terrestris</i> , same.	Same tree on orange stock below bud union.	5 by 2.....	2
7	<i>Pythiacystis citrophthora</i> from lemon fruit, California.	Lemon bark above bud union.	30 by 18 (Pl. 6, C).	3
8	<i>Pythiacystis citrophthora</i> , same	Orange bark below bud union.	46 by 18 (Pl. 6, C).	2
9	<i>Phytophthora terrestris</i> , from pomelo bark, Palmetto, Fla.	Lemon bark above bud union.	20 by 5 (Pl. 6, E).	3
10	<i>Phytophthora terrestris</i> , same.	Orange bark below bud union.	25 by 9 (Pl. 6, E).	3
11	<i>Phytophthora terrestris</i> from orange bark, Cuba.	Lemon bark above bud union.	6 by 5 (Pl. 6, D).	2
12	<i>Phytophthora terrestris</i> , same.	Orange bark below bud union.	23 by 6 (Pl. 6, D).	3
13	<i>Phytophthora terrestris</i> from tangelo, Isle of Pines.	Lemon bark above bud union.	19 by 5 (Pl. 6, E).	3
14	<i>Phytophthora terrestris</i> , same.	Orange bark below bud union.	23 by 8 (Pl. 6, B).	2
15	Control (cut without inoculation).	Lemon and orange bark ..	(Pl. 6 A) ..	0

<sup>1</sup> 0=none; 1=slight; 2=medium, 3=copious.

TABLE XII.—Comparative effects of different "strains" of *Pythiacystis citrophthora* and *Phytophthora terrestris* in inoculations on trunks of 20-year-old Lisbon lemon trees at Santa Paula. Inoculations, April 9, 1914; examination, August 14, 1914

Experiment No.	Original source of cultures.	Organism.	Size of diseased area.	Gum formation. <sup>1</sup>
			Cm.	
1	Lemon tree, Santa Paula, Calif. . .	<i>Pythiacystis citrophthora</i> .	23 by 10....	3
	Lemon tree, Whittier, Calif. ....	....do.....	8 by 5.....	2
2	Orange tree, Lindsay, Calif. ....	<i>Phytophthora terrestris</i> .	2.5 by 1.3..	1
	....do.....	....do.....	8 by 4.....	2
3	Pomelo tree, Palmetto, Fla. ....	....do.....	6 by 2.5....	2
4	Orange tree, Cuba .....	....do.....	10 by 5....	2
5	Tangelo tree, Isle of Pines.....	....do.....	....	2

<sup>1</sup> 1=slight; 2=medium, 3=copious.

The lesions produced by the inoculations with the two fungi were identical in general appearance. The characteristic manner of killing the bark, the formation of the outer noninvaded gummy zone, and the exudation of gum were the same with both fungi. Cuts without inoculation healed without producing disease.

In addition to these inoculations, a number of other comparative inoculations with pure cultures of *Pythiacystis citrophthora* and *Phytophthora terrestris*, using fruits affected with the two fungi, were made on the large main roots and at the crown of old orange trees. In nearly all the successful inoculations of this kind, lesions resulting from the two species of fungi could not be distinguished clearly one from the other and resembled true mal di gomma or footrot lesions as they occur in Florida. Most of the lesions became self-limited in three to four months on old orange trees, after enlarging to a maximum of 8 by 10 cm., but some made with *Pythiacystis citrophthora* progressed longer and developed large patches, in one case spreading 25 cm. and killing a large main root.

#### CONTROL OF PYTHIACYSTIS GUMMOSIS

More rational control methods based directly upon a knowledge of the cause and development of the disease and upon the results of many experiments<sup>10</sup> growing out of this knowledge now became possible. Previous control methods, though based largely on a different explanation of the cause, were partially successful because they frequently removed entirely the conditions contributing to infection and development of the disease, which conditions were formerly thought to be the sole cause.

The most successful method of prevention for new plantings is obviously the use of the resistant sour-orange stocks budded high. This method was employed in Italy after the orchards were killed out by gummosis and has since been used largely in Florida and California. To prevent gummosis on susceptible stocks or on low-budded trees, the method now in common use in California on heavy soils is to pull back the soil from the base of the trunk, thus exposing the top of the first main roots and making a circular ridge to exclude irrigation water from standing in contact with the trunk of the tree. As an added preventive on soils especially subject to gummosis, the base of the trunk is painted with Bordeaux paste or other noninjurious fungicide. In one experiment with Bordeaux used in this way on about 100 acres of lemons the number of new cases decreased from 123 in 1912 to 16 in 1914. By a similar treatment in another locality with 23,837 lemon trees the number of new gummosis outbreaks decreased from 727 in 1912 to 113 in 1914 (from 3.5 to 0.5 per cent). In a third experiment with 560 lemon trees, one-half of which was treated, 9 per cent of those receiving Bordeaux spray two previous years developed disease, against 21 per cent of those not so sprayed.

The treatment of trees after they are diseased consists in removal of the bark tissue from the invaded zone and the application of a fungicide to kill out the fungus in small bits of tissue possibly left behind and to prevent reinfection (Pl. 3, B-D). No attempt need be made to cut beyond the large outer gummy zone, since it has been shown that this does not contain the invading parasite and will recover rapidly after the inciting cause for the gumming has been destroyed.

<sup>10</sup> The detailed results of experiments in prevention and control will be presented in a bulletin from the University of California Agricultural Experiment Station.

## PART II.—GUMMOSIS DUE TO BOTRYTIS CINEREA AND OTHER FUNGI

## BOTRYTIS GUMMOSIS

## INTRODUCTION

*Botrytis gummosis* differs from *Pythiacystis gummosis*, discussed in Part I of this paper, in that it causes softening of the invaded bark in the early stages and produces conidiophores and spores in damp, cool weather. In the later stages the outer layer of bark is killed and becomes dry and hard much in advance of the inner layer, while there is a greater tendency than in *Pythiacystis gummosis* for the tree to renew the bark underneath the dead hard layer and there is usually also a less copious flow of gum. *Botrytis gummosis* is confined in California almost exclusively to lemon trees growing in the coastal regions and is usually seen on trees more than 10 years of age. This disease should not be confused with a desquamated-bark (shellbark) condition in which the outer bark on old lemon trees dies, cracks, and breaks away in longitudinal strips, a condition which is somewhat similar to that frequently brought about in the later stages of *Botrytis gummosis*. Neither disease should be confused with psorosis (scaly-bark) of sweet-orange trees.

The causal fungus, *Botrytis cinerea* Lk., depends to a much greater extent than does *Pythiacystis citrophthora* Sm. and Sm. on wounds or other conditions predisposing the bark to attack.

The writer's attention was first called to this type of gummosis early in February, 1912. After a period of moist, cool weather, patches of bark 15 to 30 cm. long and half as wide presented the gray furry appearance characteristic of the fruiting bodies of *Botrytis cinerea*. In a later survey of the Citrus districts of California, *Botrytis cinerea* was usually found associated with this type of gummosis and was isolated from a large number of diseased lesions. *Penicillium roseum* Link,<sup>1</sup> *Fusarium* sp., and several other fungi were also frequently conspicuous.

The important question at once arose, whether any of these fungi might break down sound tissue and initiate conditions leading to disease and gum formation or whether gumming and associated death of the bark might result primarily from injuries or other contributing conditions, the fungi playing only a minor or secondary part, as had previously been assumed. It seemed advisable, therefore, to attempt to answer this question experimentally.

## INOCULATION EXPERIMENTS

## Preliminary Tests

On March 7, 1912, at Santa Paula, a preliminary set of rough inoculations was made with diseased tissue and with fungi taken directly from the surface of the bark.

Sixteen Lisbon lemon trees 18 years old, on sweet-orange stocks, were used for the inoculation. The portion of the bark to be inoculated was washed first with water and then with alcohol, and the flame of an alcohol lamp was quickly passed over the surface. A cut about 3 cm. long was made through the bark with a heavy sterilized knife. Into these cuts the materials for inoculation were inserted. The cuts were made at varying

<sup>1</sup> Determination of this species was kindly made by Dr. Charles Thom.

distances, ranging from 5 to 60 cm. above the "bud union." With every inoculation a similar cut, to serve as a control, was made in the opposite side of the same tree. A sheet of paraffined paper was then tied against the surface of the bark over each cut and fastened with wax at the upper edge to exclude rain and dust.

The different kinds of inoculum and the number of trees were as follows: (1) Small pieces of sound bark and wood, four trees; (2) bit of diseased bark, two trees; (3) small bits of wood permeated with gum from outer gummous zone, two trees; (4) small portion of exuded gum, one tree; (5) bits of dead bark containing *Penicillium roseum* Lk., two trees; (6) hyphae and spores of *Botrytis cinerea*, taken from rotting diseased bark, three trees.

None of the inoculations with sound wood or bark, gum-filled wood from the outer gummous zone, or exuded gum produced any gumming or development of disease. All the inoculations from diseased tissue, however, showed gum exudation within 2 to 4 months, and in one case a lesion 8 by 15 cm. developed in 9 months. One of the inoculations with *Penicillium roseum* showed slight gum exudation in 4 months without development of a lesion, but the other cut healed without gumming. All three of the *Botrytis* inoculations resulted in gummosis of the same type as that from which the fungus was obtained. In one of them slight softening of the bark was noted in 10 days. A softened area of bark which measured 8 by 2.5 cm. in 50 days (with fruiting of *Botrytis* on the surface) had increased to 10 by 4 cm. in 4 months, with copious exudation of gum some of which was exuding through cracks 50 cm. directly above the cut in the outer noninfected gummous zone. About 1 year after this inoculation the space over which the bark was dead to the wood measured 10 by 2.5 cm., and this was surrounded by an irregular area over which only the outer bark was killed, making the entire area 30 by 27 cm. *Botrytis* was fruiting at various places over this area.

A second set of experiments was then carried out. These were planned to test the influence of various kinds of wounds, the influence of obstructions in the sap current, and finally, the influence of continued pressure upon the bark. These experiments were made in July, 1912, on sound trunks of 19-year-old Lisbon lemon trees at Santa Paula. The following methods were used both with and without contamination with pure cultures of *Botrytis cinerea*: Cuts vertically or horizontally through the bark, auger holes with and without glass or wooden plugs, bruises made by light and heavy blows from hammers, wounds made by slicing off both thin and thick layers of bark, wounds made by entirely cutting off large areas of the bark and so forth. Constant pressure was also exerted against the bark with wooden blocks tightened by means of screws in an iron collar.

It was difficult to make these inoculations or injuries in tree trunks in the open and keep them absolutely free from organisms. In attempting to overcome this difficulty the following method was adopted for the most important of these experiments. A cloth hood to be tied to the tree trunk was made by fastening a piece of fumigation-tent cloth to a wooden barrel hoop severed on one side. Strings were fastened to the cut ends of the hoop and to the corners of the cloth opposite the hoop. The hoop was allowed to hang down. The upper strings were tied to the trunk of the tree just below the branches and the hoop was fastened

below so as to cause the cloth to flare outward and thus protect the wound from falling dust and excessive currents of air. In order to prevent dust in dry weather, the under surface of the hood, the trunk, and the soil around the base of the tree were sprayed with water. The area of bark to be experimented on was washed, first with water and then with alcohol, and quickly flamed with an alcohol lamp. The augers, hammers, knives, or other instruments used were sterilized either by heat or by alcohol which was allowed to evaporate from their surfaces. Either grafting wax or glass slides sealed with putty were used as a covering in most of the experiments.

There was no gum exudation or development of disease in connection with any of the experiments with which attempts were made to keep the bark free from contaminations. With the experiments in which spores of *Botrytis cinerea* were used as a contamination, however (except where the bark was not injured) gum exuded, and in most cases typical *Botrytis* gummosis lesions developed around the place of injury.

The results of these experiments appeared to show that injuries were not sufficient in themselves to induce gum diseases. When the injuries were contaminated with pure cultures of *Botrytis cinerea*, however, under the same conditions, gum formation and death of the tissue were readily produced.

#### Inoculations with Pure Cultures of *Botrytis cinerea*

Many inoculations have been made with pure cultures of *Botrytis cinerea* and the following is typical of the results.

On July 12, 1912, spores and sporophores of *Botrytis cinerea* were inserted in a cut 2.5 cm. long, on the trunk of an 18-year-old lemon tree at Santa Paula. The wound was then covered with oiled paper. Gum was exuding rapidly by July 20 and continued to do so until a diseased area 4 by 5 cm. was noted on August 22. On November 21, there was a softened, dead area of bark, 15 by 5 cm., which on February 14, 1913, was 23 by 8 cm. in size and on June 27, 1913, 28 by 10 cm., with new gum, and *Botrytis* fruiting on a part of the surface. On July 28, 1913, the main area was 32 by 10 cm., and a number of smaller areas were scattered over the same side of the trunk. On scraping the trunk for treatment at this time, it was found that only a small area of bark was killed through to the wood, only the outer cortical layer of the remaining part being dead. Bordeaux paste was applied to one lateral half of the scraped portion. On November 25, 1913, the disease was seen to be arrested on the portion treated with Bordeaux, but further dying of outer bark had taken place on the other portion. A similar cut without inoculum made on the opposite side of same tree healed without gumming or any other apparent effect.

In all, about 40 inoculations with pure cultures of *Botrytis cinerea* were made on lemon trees, most of which resulted in gummosis of the type represented by the previous example. The general results of a number of these are given in Table XIII.

TABLE XIII.—*Inoculations with pure cultures of Botrytis cinerea, on 18-year-old lemon trunks except where noted*

Ex- peri- ment No.	Date of inocu- lation.	Host.	Gum- ming	Botrytis fruiting <sup>1</sup>	Cracked outer bark area <sup>1</sup>	Date of examina- tion	Area killed, outer bark.
1	July 12, 1912	Lemon	x	x	x	July 28, 1913	Cm 32 by 8
2	....do.....	..do..	x	o	x	Mar. 9, 1916	30 by 8
3	Aug. 23, 1912	..do..	x	x	x	....do.....	30 by 8
4	Dec. 23, 1912	do..	x	x	x	....do.....	10 by 8
5	Feb. 20, 1913	..do..	x	x	x	....	
6	Mar. 14, 1913	..do..	x	x	x	Sept. 3, 1914	20 by 5
7	July 13, 1913	..do..	x	o	x	Apr. 7, 1914	Very small.
8	....do.....	..do..	x	o	x	....	
9	....do.....	Orange	o	o	o	Feb. 6, 1915	No effect.
10	....do.....	Lemon..	x	....	x	Apr. 2, 1914	Very small.
11	....do.....	do..	x	....	x	....do.....	Do.
12	....do.....	..do..	x	....	x	....do.....	Do.
13	....do.....	do..	x	....	x	....do.....	Do.
Control cuts for each experi- ment.....			o	o	o		

<sup>1</sup> x = positive; o = negative.

With most of these inoculations made on old lemon tree trunks such effects as the softening and death of the bark through to the wood in the earlier stages, the appearance of fruiting bodies, formation of gum, and the subsequent development of an area of dead outer bark surrounding the initially invaded area and finally of an outer noninvaded gummous zone were found to be identical with those of cases of the disease occurring naturally.

Further inoculations into cuts on young bark of several species of Citrus and of deciduous trees are reported in Table XIV.

TABLE XIV.—*Inoculations with spores and mycelium from pure cultures of Botrytis cinerea on young trees of Citrus, and other hosts*

I. INOCULATED JULY 2, 1913; EXAMINED AUGUST 11, 1913

Experi- ment No	Host.	Kind of tissue.	Results.
1	Valencia orange	Young branch .....	Gumming slightly.
2	Sour orange .....	Small branch .....	Healing without gumming.
3	Lemon .....	Hardened bark .....	Cut filled with gum and healing back of it.
4	....do.....	Young twig .....	Gum more copious than last; not healing.
5	....do.....	Same as above without inoculum.	Healing without gumming.
6	Almond .....	Stem of young tree .....	Gumming with small portion of bark killed at cut, then healed.
7	Peach .....	....do.....	Much gumming. Killed slightly on each side of cut. Bark area 6 cm. long in wood.
8	Plum (Burbank).	....do.....	Gumming at first and then healing.
9	Pear .....	....do.....	Healed with enlarged scar.
10	....do.....	Same as above without inoculum.	Healing perfectly.



TABLE XIV.—*Inoculations with spores and mycelium from pure cultures of Botrytis cinerea on young trees of Citrus, and other hosts—Continued*

II. INOCULATED APRIL 17, 1913; EXAMINED MAY 20, 1913

Experiment No.	Host.	Kind of tissue.	Results.
11	Common lemon...	Trunk 3 years old.....	Bark soft and decaying over areas of 0.5 by 1.5 inches. Much gum exuding.
12	Sweet orange.....	.....do.....	Bark slightly killed. Slight internal gumming.
13	Same as above without inoculum.	.....	No effect on bark.

III. INOCULATED FEBRUARY 6, 1915; EXAMINED MAY 18, 1915

14	Common lemon...	Branch about 2 years...	Gumming and fruiting of Botrytis on killed bark.
15	Rough lemon...	Trunk 2 years old.....	No effect.
16	Sour orange.....	.....do.....	Do.
17	Same as above without inoculum.	.....	Do.

With comparatively young common lemon, peach, and almond trees these inoculations with spores placed in cuts resulted in the formation of considerable gum. The gum was slight in sweet orange and plum. No gum was induced in sour orange, rough lemon, or in any of the control cuts except on peach. The death of the bark was slight and unimportant in extent except in the common lemon and peach.

In general the results of all these experiments showed that the effects of *Botrytis cinerea* were quite different from those of *Pythiacystis gummosis*. This fungus, unlike *Pythiacystis citrophthora*, was not able to make an entrance except through some wound or defect in the bark, and it was not able to progress so rapidly in killing the bark through to the wood. After a limited portion was killed to the wood, however, a larger surrounding area was involved, in which only certain outer layers of bark tissue were killed, leaving the cambium alive and capable of renewal. There was also in the *Botrytis* gummosis an outer gummous zone beyond the invaded zone, but this was usually less extensive and less rapidly formed than in *Pythiacystis* gummosis. Other conditions being equal, there was usually somewhat less gum formation in *Botrytis* gummosis than in *Pythiacystis* gummosis.

The fungus was reisolated from the softened invaded area of a large number of these lesions. Attempts to isolate the fungus from the outer gummous zone failed, just as they did in *Pythiacystis* gummosis. Only rarely was *Botrytis* isolated from the area where the outer bark was dead and hard. Cultures showed that this outer dead cortical layer following *Botrytis* inoculations is, under such conditions as prevail at Santa Paula, rapidly occupied by species of *Alternaria*, *Cladosporium*, *Penicillium*, *Colletotrichum*, *Fusarium*, and other fungi as well as bacteria.

## FACTORS FAVORING BOTRYTIS GUMMOSIS

Many contributing conditions favor the occurrence and the severity of this disease. These are similar to those which favor *Pythiacystis* gummosis, mentioned in Part I of this paper, to be discussed in more

detail in a subsequent bulletin of the California Agricultural Experiment Station.

Injuries of various kinds to the bark, not only near the soil but anywhere on the trunk or large branches, may lead the way to infection and development of *Botrytis gummosis* when the conditions of moisture and temperature are also favorable. This disease is frequently severe on living tissue of trees that have been injured by frost. The fungus may become established in such trees first in a small portion of dead or dying tissue and then advance rapidly into tissue which appears to be sound.

A desquamated condition of bark, fairly common on old lemon trees in the California coastal regions, is also frequently accompanied by *Botrytis gummosis*. It furnishes dead outer bark tissue from which the fungus may advance. This desquamated condition is similar in appearance to that which usually follows inoculations with *Botrytis cinerea* on sound tree trunks and with which it is often confused, but which is thought to be due to other causes.

The previous use of "neat's-foot oil" in the treatment of gummosis also encouraged the growth of this fungus. Lemon trees previously treated at Santa Paula by scoring the bark and painting with neat's-foot oil were observed in February and March of 1912 to have their trunks fairly well covered with a gray coating consisting of the sporophores and spores of *Botrytis*. The bark on these trees was found to be in various stages of soft decay with the exudation of large masses of gum. Experiments also showed that this fungus grew better on bark treated with neat's-foot oil either before or after infection by the organism than on bark free from this oil. More recently the application of neat's-foot oil to Citrus trees has been largely given up, and the more severe stages of this disease, such as were previously seen, have not been observed.

#### CONTROL

The principles governing the control of this type of gum disease are similar to those of *Pythiacystis gummosis*: (1) The prevention of infection by avoiding injuries, and the use of fungicides, and (2) if that is not done, the elimination of the invaded tissue. As the result of many different experiments in which growers took a prominent part, a method consisting largely of scraping off the outermost layers of bark proved to be best adapted for treatment of this disease. The portion where the bark is totally killed is cut away, but beyond this, where only the outer layers of bark are dead, these outer layers only are scraped off, leaving the live inner layer next to the cambium intact. To prevent further invasion of bark it is usually found necessary to scrape the sound bark several inches beyond the margin of the affected region. A sharp curved tool made on the principle of a box scraper is in general use for this purpose. Where both types of gummosis are present on the same trees, as is frequently the case, this method is applicable to the combined lesions produced. The cut or scraped portions are then painted with a fungicide. Bordeaux paste, and some of the coal-tar products which contain only the heavier oils, have given good results in experimental work and in practice. A discussion of the experiments on which these control methods are based will be reported in a bulletin of the California Agricultural Experiment Station.

## SCLEROTINIA GUMMING, DUE TO SCLEROTINIA LIBERTIANA

This disease, usually of minor importance, occasionally has been found associated with rapid dying of bark on the roots and trunks of Citrus trees growing in damp, cool situations, especially after periods of severe frost. The bark is at first soft, as if from an attack by *Botrytis cinerea*. Though the fungus usually advances more rapidly than Botrytis, it is soon checked, and callus begins to form as soon as gum accumulates. Later, as the bark dries, it is left in shreds and large black sclerotia are found within and under this bark. Its effect on Citrus twigs has been described by C. O. Smith (54), who refers to the gumming usually accompanying its attack. It appears to infect the young growth, usually at the blossoming period and frequently extends back into larger branches.

When found on the trunk or roots, observations have indicated that previous injury of the bark was usually necessary for the entrance of the fungus. It has frequently been found on young trees following frost injuries, apparently advancing from frost-injured tissue into tissue not killed by frost. It has been observed on a 20-year-old lemon tree where all the roots had been infected, probably from injuries made in digging about them and placing vetch straw near the crown in damp, cool weather. An old seedling orange tree was also observed with the bark on one side of the trunk killed by the fungus, which had apparently gained entrance through a slight sunburned area and had advanced into the live bark for some distance.

In order to obtain some idea of the ability of *Sclerotinia libertiana* to break down sound bark and induce gumming, several inoculations with pure cultures were made on healthy lemon trees, of which the following examples may be given:

On February 20, 1913, a bit of mycelium from a pure culture was inserted into a vertical cut in the bark of an 18-year-old Lisbon lemon tree. A similar cut to serve as a control was made on the opposite side of the same tree. These were then covered with oiled paper. The cut not inoculated healed normally, but the bark adjacent to the inoculation was soft on one side within 4 weeks. In 8 weeks there was a dead, soft area 15 by 8 cm., which had increased to 22 by 9 cm. in 11 weeks with a white mycelium conspicuous over a smaller area of 13 by 5 cm. About 4 months after inoculation (June 27, 1913) the area of dead bark measured 30 by 14 cm. and increased to 45 by 15 cm. by July 28, 1913, after which it soon ceased enlarging and became self-limited. The gum began to appear in 6 weeks; its rate of formation increased rapidly and reached a maximum about 3 months after inoculation. It ceased to increase about 5 months after inoculation. The sclerotia were seen to have formed in flat plates under and within the bark in 4 months and appeared to be alive for at least 2 years, but no subsequent activity was apparent in this lesion for a period of at least 3 years. The killed bark showed the characteristic shredded appearance. Without treatment, there was a complete stoppage of further invasion by the fungus.

A 2-year-old lemon tree was similarly inoculated 1 inch above the bud union on February 25, 1913. By May 7, 1913, the tree was girdled and showed gumming, the bark being killed 6 inches upward and 1 inch downward to the bud union. The invasion did not advance into the sour-orange tissue of the stock. The foliage at this time showed no effect, but by June 27, 1913, the leaves were wilted. In both these cases similar

cuts not inoculated, made as controls, healed without visible injury to the tree.

As indicated by these inoculations, the attack of *Sclerotinia libertiana* may be very severe and its progress very rapid for a comparatively short time, and then it may be quickly halted. If the tree trunk attacked is small, girdling and death may result, while on a large trunk with not more than one point of infection, self-recovery may take place. Observation shows that the halting of the invasion is usually coincident with the formation and exudation of considerable quantities of gum.

#### EXPERIMENTS TO TEST THE POSSIBLE RELATION OF OTHER ORGANISMS TO GUMMOSIS

A number of other organisms, most of which were found commonly on dead or decaying bark of Citrus trees, were used in inoculation experiments to ascertain their relation, if any, to gummosis.

The results of inoculations with various cultures of *Fusarium* sp., commonly found associated with *Pythiacystis* gummosis, have already been presented on page 205. The effect of *Fusarium* alone was insignificant. The death of the bark at the inoculated cuts was inconspicuous, and only slight death of the outermost layer of bark resulted over small areas. Only part of the inoculations produced gum, usually in small quantity.

*Penicillium roseum* was frequently found on dead bark affected with gummosis. The results of some of the inoculations with pure cultures of this fungus are presented in Table XV.

TABLE XV.—Inoculations with *Penicillium roseum* at Santa Paula

Experiment No.	Date of inoculation	Kind of inoculation.	Gum.	Initially killed bark at inoculation point. <sup>1</sup>	Cracking of outer bark. <sup>1</sup>
1	July 12, 1912	Vertical slit 2 cm. long, covered with oiled paper . . . . .	2	1	2
2	Aug. 23, 1912	Vertical slit . . . . .	2	1	0
3	Feb. 21, 1913	Vertical slit not protected . . . . .	2	1	2
4	do. . . . .	Controls on same trees as above . .	0	0	0
5	Feb. 20, 1913	Vertical slit not protected . . . .	1	1	0
6	do. . . . .	Control on same tree . . . . .	0	1	2

<sup>1</sup> 0=none, 1=slight, 2=medium.

Gumming was induced in all the cuts inoculated with *Penicillium roseum* in Table XV. The dead bark adjacent to the inoculated cuts was slight in amount. The outer layer of bark surrounding two of the inoculated cuts and only one of the controls died and cracked.

Inoculations were made with a number of other organisms, as shown in Table XVI. All of these were found on Citrus trees except *Coryneum berynkii* and *Pseudomonas cerasius*.

TABLE XVI.—Inoculation with miscellaneous organisms

Experiment No.	Date of inoculation.	Variety and age of host.	Organism.	Gumming. <sup>1</sup>	Death of bark. <sup>1</sup>
1	Nov. 23, 1912	Lemon, 19 years..	<i>Alternaria</i> sp. from injured fruit.	1	1
2	Feb. 25, 1913	Lemon, 2 years....	<i>Alternaria</i> sp. ....	0	1
3	May 24, 1912	Lemon 16 years...	<i>Coprinus atramentarius</i> , mycelium.	0	0
4	.....do.....	.....do.....	Spores of same.....	2	1
5	July 13, 1913	Lemon, 19 years...	<i>Coprinus atramentarius</i> mycelium.	2	1
6	May 24, 1912	Lemon, 16 years...	<i>Hypholoma</i> sp. spores..	1	0
7	Aug. 3, 1912	Lemon, 16 years (2 trees).	<i>Hypholoma</i> sp. mycelium.	1	0
8	Sept. 25, 1912	Lemon, 19 years...	<i>Cladosporium</i> sp.....	0	0
9	.....do.....	.....do.....	<i>Rhizopus</i> sp.....	0	0
10	Sept. 4, 1913	Lemon, 18 years...	<i>Penicillium digitatum</i> ...	0	0
11	Aug. 3, 1912	Lemon, 16 years...	<i>Diplodia</i> sp.....	2	1
12	Nov. 23, 1912	Lemon, 19 years...	<i>Spegazzinia ornata</i> .....	0	0
13	July 13, 1913	.....do.....	<i>Coryneum berynkii</i> from peach.	2	1
14	.....do.....	Orange stock, same tree.	.....	1	0
15	July 12, 1912	Lemon, 19 years...	<i>Pseudomonas cerasius</i> Griffin.	0	0
16-25	.....do.....	.....do.....	10 different cultures of bacteria inoculated from bark killed by gummosis.	0	0
Controls, without inoculum to correspond with each of the foregoing experiments.				0	0

<sup>1</sup> 0 = none, 1 = slight, 2 = medium

The results show that several different organisms are usually capable of inducing the formation of a small quantity of gum and a limited amount of injury to cells adjoining a wound when inserted into cuts on sound tissue but are without any noticeable effect in producing definite diseases. This gum formation or death of tissue was, in these cases, as well as with *Fusarium* sp., and *Penicillium roseum*, insignificant in amount as compared with that produced in cases of either *Pythiacystis* or *Botrytis* gummosis.

### PART III.—GUM FORMATION AND ITS RELATION TO THE DEVELOPMENT OF DISEASES

#### INTRODUCTION

In Parts I and II of this paper the relation of certain fungi to definite diseases in which gum formation was a conspicuous feature has been discussed. It was shown experimentally that the most destructive types of gum diseases on Citrus in California in which there is progressive dying of tissue over large areas are due to fungus invasion. Fungi, however, are not necessary to mere gum formation, since it has been shown that other agencies, such as chemical injections, may induce gum formation in the absence of microorganisms.

It is the purpose of Part III to discuss more especially the process of gum formation itself, the conditions facilitating its formation, and its relation to the development of diseases.

#### NATURE AND ORIGIN OF THE GUM

Although gum appears to be formed in many other plants continuously as a "normal" process, it is usually not formed in Citrus except under the influence of stimuli more or less injurious to the tree. Citrus gum is similar to cherry gum and gum arabic, the latter of which is known to retain the nature of an acid, the molecule being composed of a number of sugar residues grouped about an acid nucleus in such a way as to leave the acid group exposed.

These gums differ from resins in being for the most part soluble in water and insoluble in alcohol, while the opposite is true of the resins. This solubility in water also distinguishes them in a rough, imperfect way, from mucilages, which have a more slimy consistency and merely swell up in water. There are, however, all gradations between gums so defined and the mucilages. As to origin and chemical composition, the distinction between the vegetable gums and some of the mucilages can probably not be clearly maintained. These exuded gums, however, should probably be distinguished from a hard, vitreous gumlike substance known as wound gum, which Higgins (40) refers to as occurring in the wood elements in the vicinity of wounds as a general phenomenon in woody plants. It differs from the ordinary gums in not swelling in water and in giving the lignin test.

Although there has been considerable difference of opinion as to the direct origin of gums and mucilages in plants, most investigators, including Grafe-Wien (35), Czapek (36), and Lloyd (45), have concluded that the gums like those represented by gum arabic, cherry gum and Citrus gum, are derived mainly from the cellulose walls. Greig-Smith (36), however, has reported the formation of gums of this nature by the direct action of bacteria on certain culture media containing no cellulose. Gum related to the dextrans also has been obtained by the action of *Bacillus radicolica* on culture media containing sacchrose by Buchanan (12), who concludes that this gum arises from the diffuent wall of the bacterial cell. MacDougal, Richards, and Spoehr (47) have pointed out that in cacti the formation of mucilagelike gum results from the dehydration of sugars and condensation of their products as a normal process in this plant.

In Citrus, however, the gum appears to arise mainly from the hydrolysis of the cellulose walls. The initial gum originates, according to Butler (13) and Floyd (31), between the medullary rays usually in thin-walled xylem cells newly laid down. The protoplasm becomes more granular, the cells round out, separate from each other, and dissolve from the outside inward, and the space is finally occupied by a mass of gum. It may also be derived apparently from the breaking down of other and older tissues in connection with some of the severe types of Citrus gummosis.

#### PHYSICAL EFFECTS AND GUM FORMATION

##### MECHANICAL INJURIES

The writer has not been able to induce gum exudation on healthy Citrus trees by mechanical injuries alone, provided these injuries are

kept clean and reasonably free from contamination with microorganisms or unusual chemical substances.

A number of experiments were carried out to test (a) the influence of various kinds of wounds, (b) the influence of obstructions in the sap current by insertion of substances such as glass or wooden plugs, and finally (c) the influence of continued pressure upon the bark. Most of these experiments were performed with and without contamination with spores from pure cultures of *Botrytis cinerea* and are shown in Table XVII.

TABLE XVII.—Experiments with injuries with and without contamination, started in July, 1912, on sound trunks of 18-year-old lemon trees at Santa Paula

Treatment of tree.	Number of experiments.	
	Not inoculated.	Inoculated.
Tangential slice of outer bark cut off and microscopic slide fastened over with putty.....	2	2
Surface of bark lightly scraped and covered as above.....	1	1
Bark not injured but covered as above.....	1	1
Long slits made through bark and covered with grafting wax.....	1	1
Areas of bark about 8 by 4 cm. cut away and covered with cold liquid grafting wax.....	5	5
Bark on trunk injured with heel of heavy boot, as if climbing tree.....	1	1
Bark injured by throwing wire coal basket against trunk.....	1	1
Bark injured by blow from blacksmith's hammer and covered with wax.....	2	2
Auger holes 1.3 to 2 cm. deep and 1 to 1.5 cm. in diameter filled with glass tubes and sealed in with wax.....	5	2
Pressure exerted against wooden blocks, on opposite sides of tree, by means of screws in an iron collar.....	3	.....

On none of the injuries shown in Table XVII, kept uninoculated, was there any gum exudation or development of the disease. All such injuries healed in the usual way. With all the experiments in which spores of *Botrytis cinerea* were used as a contamination, however, except where the bark was not injured and one of those with glass tube used as a plug, gum exuded and in most cases typical *Botrytis gummosis* lesions developed around the place of injury.

The results of these experiments appeared to show that injuries in themselves were not sufficient to induce gum formation. When the injuries were contaminated with pure cultures of *Botrytis cinerea*, however, under the same conditions, gum formation and death of the tissue readily took place. Other injuries of various kinds also failed to induce gumming in most cases even when no means were provided to keep the wounds free from chance contaminations from the air or from water during rains. These same wounds, however, when purposely infected with *Botrytis cinerea* or other injurious organisms resulted in gum formation. Mechanical pressure and obstructions placed in the conducting system likewise failed to induce gum in these lemon trees. In previous experiments conducted by Fawcett (22), clean injuries made by cutting through the bark of *Prunus persica*, *Prunus umbellata*, *Prunus serotina*, *Laurocerasus caroliniana*, *Xanthoxylum americanum*, and *Rhus glabra* in Florida failed to induce gum, but when similar injuries in all these species were inoculated with *Diplodia natalensis*, gum formation resulted.

In *Prunus* and other plants, however, mechanical injuries in themselves have been considered as sufficient to induce gum formation. Butler (13) was successful in inducing gum formation on peach, cherry, and plum by bruising the bark with a mallet. No mention is made of similar experiments on Citrus.

The injuries on Citrus made by a number of insects are frequently followed by gumming, usually slight in amount. Small drops of gum may form on fruit at points of injury produced by the orange tortrix (*Tortrix citrana*), and on small tree trunks and limbs by grasshoppers, katydids, and other insects. To what extent this gumming may be due to secretions of the insects and to what extent to the entrance of micro-organisms at the time of injury is uncertain. Our negative results from mechanical injuries on Citrus kept sterile and free from chemical stimuli would indicate that this gumming was probably not due to the injury or wound in itself.

#### BURNING

In the author's experience, burning was not in itself any more effective than mechanical injuries in producing gum formation in Citrus. The flame of an alcohol lamp was held against the trunk of young Citrus trees until the bark was severely injured, but no gumming resulted. Examination of many Citrus trees that have been accidentally injured by fire has shown that bark may be killed on one side of twigs and branches without resulting in gum formation. Severe injuries from sunburning on Citrus have also been observed to be free from gum formation, provided they are not followed by invasion of parasitic or wood-rotting organisms. Butler (13), however, reports the production of gum on young shoots of *Prunus* by burning with a hot iron.

#### FREEZING

Freezing has also been considered a frequent cause of gum formation. Observations of hundreds of Citrus trees in California in all stages of injury from frost have not indicated that freezing in itself is an important factor in initiating gum formation. Frost injury, however, may frequently be followed by invasion of organisms such as *Botrytis cinerea*, *Sclerotinia libertiana*, or other fungi, which after becoming established in the injured tissue may advance rapidly and induce gumming in tissue apparently sound.

#### DESICCATION

Drying or partial desiccation of cells in plants has been suggested as an important factor in gum formation. As the result of a number of controlled experiments with species of *Prunus*, drying of the tissue was considered by Higgins (40) as an important factor in the acceleration of gum formation. Sorauer (59, p. 708) cites Martin as mentioning the action of dry desert winds of autumn and winter as having a relation to gum formation in *Acacia senegal*. It has been pointed out by MacDougal, Richards, and Spoehr (47) that when *Opuntia* plants are subjected to long periods of drought, resulting in partial desiccation, the sugars, which have a low water-holding capacity, are converted into the pentosans or mucilages, which have a high imbibition capacity.

That this factor of partial desiccation operating alone can not account for excessive gum formation in Citrus seems to be indicated by the fact that such gum formation does not necessarily follow injuries from frost



or sunburn, nor does it necessarily result from drying of tissue in wounds if these are kept uncontaminated by microorganism or chemical stimulants.

Some of the results of the investigations reported in Parts I and II of this paper have a bearing on this subject. It was shown that gum formation could be induced in cuts inoculated by *Pythiacystis citrophthora* or *Botrytis cinerea* where these were covered with grafting wax or waxed paper, which presumably prevented undue loss of water from the surface. It is, of course, possible that dehydration of the cells invaded by the parasite might have taken place even in this case, without loss of water through the surface, by changes in internal conditions by which water was lost to the surrounding tissues. With *Pythiacystis* gummosis excessive water in the soil about the trunks, rather than drying or partial desiccation, is the condition favorable for the invasion of the bark by the causal organism that is instrumental in bringing about gum formation.

While partial desiccation in itself does not appear to be an important factor in initiating gum formation in Citrus, it is probably one of the factors in producing an acceleration of the process when the other necessary factors are already in operation. It has been noticed also that when a lesion is developing and gumming moderately, the coming of dry weather is frequently followed by increased exudation of gum for a brief period. If the weather is sufficiently dry, however, the enlargement of the invaded zone often ceases, probably due to the dying out of the parasite, and the rate of gum formation soon decreases to zero. It appears that in *Pythiacystis* gummosis, at least, the conditions of desiccation that tend to increase the gum flow are frequently those that hinder or kill out the causal agent, while furnishing a temporary stimulus to increased gum formation. If, as seems highly probable, partial desiccation of the cells is one of a group of factors which favor excessive gum formation in Citrus, it seems clear that other more important influences or stimuli must precede or accompany it.

#### CHEMICAL STIMULI AND GUM FORMATION

Many substances have been reported as inducing gum formation in Citrus. A study of gum formation was made by Floyd (31) by the introduction of 28 organic and inorganic substances into young Citrus trees. Thirteen of these induced gum formation. Most of them were acids, alkalies, or salts of heavy metals. In general, the salts of the heavy metals brought about the greatest amount of gumming. The gum formation was coincident with the injury from the chemicals; the gum was small in amount and was formed in proximity to the region of insertion of the chemicals except in case of some of the salts of the heavy metals. The gum originated in all cases in the live embryonic xylem tissue in regions or zones beyond the dead area produced by the chemical. None of the cuts or injuries used as controls, and not inoculated with chemicals, produced any gum formation. The dead area produced by the chemical is thus seen to be directly comparable to the invaded zone in *Pythiacystis* gummosis, and the region of chemically induced gum formation beyond this is directly comparable to the noninvaded "outer gummous zone" of the disease.

Experiments on Citrus had been made previously with a limited number of chemicals. Butler (13) induced gum formation with sul-

phuric, phosphoric, nitric, and lactic acids, and with potassium hydrate but failed to induce it with acetic acid or kerosene; and Fawcett (23) induced gum with nitric, sulphuric, acetic, citric, and phosphoric acids, copper sulphate, mercuric chlorid, and ammonium lactate but failed to induce it with carbolic acid.

Among the chemical stimuli that have been seen to result in gum formation occasionally in Citrus orchards may be mentioned: (1) liquid hydrocyanic acid spilled on the soil too near the roots of trees; (2) hydrocyanic acid gas used in fumigation; (3) spray mixtures containing copper sulphate not properly neutralized with lime, or containing other toxic substances; (4) ant poison containing arsenic.

It has not been possible, however, to duplicate entirely by chemical stimuli any of the typical maladies produced by certain organisms. While the gum formation induced by chemicals may be slight or extensive, the stimulus is soon at an end and the wound usually begins to heal promptly without the long-continued, progressive killing and gumming characteristic of *Pythiacystis* and *Botrytis* gummosis. Much confusion and apparent differences in results regarding gummosis have arisen from a failure to distinguish between the severe types of gummosis induced through the agency of microorganisms and those more temporary and usually milder types brought about by chemical or other stimuli. While the nature of the gum exuded may be the same, the manner in which the accompanying injury develops is usually very different.

#### ENZYMES AND GUM FORMATION

Only a small amount of experimental work on the rôle of enzymes in Citrus gummosis appears to have been done. The results of the writer's experiments will be presented here merely as suggestive of certain possibilities in connection with this subject.

Savastano (52) concluded that in the form of Citrus gummosis due to *Bacterium gummi*, a toxin secreted by the organism, spreads out considerable distances beyond the invaded tissue, stimulating the gum formation. In line with this view, Higgins (39) in the study of plum wilt, concluded that the fungus *Lasiodiplodia triflorae* Higgins secreted a toxic substance which reacted directly or indirectly on the zymogen of the host cells and brought about the production of a gum-forming enzym. Butler (13), on the other hand, had concluded that enzymes had no part in gum formation in Citrus or Prunus. This view, it seems, was based mainly on the origin and histology of the gum pockets and not on microchemical tests or experiments to detect the presence of enzymes. (See criticism of this view by Wolf) (65). Floyd (31) made certain microchemical tests with material taken from gum pockets on Citrus branches affected with exanthema and concluded that the enzymes present were probably hemicellulase and pectinase.

To get definite information as to the possible influence of enzymes or other filterable substances on gum formation in *Pythiacystis* gummosis, the following experiments were carried out.<sup>12</sup>

EXPERIMENT 1.—On October 1, 1912, lemon bark containing *Pythiacystis* gummosis lesions was cut from two large trees at Whittier, Calif., and 350 gm. of green material were ground, first in a meat grinder, then

<sup>12</sup> The assistance of H. D. Young, formerly of the Southern California Pathological Laboratory, in carrying out these experiments is acknowledged.

in a mortar with sand and water. One liter was decanted off and filtered through a sterilized clay filter. No organisms were found in the filtrate. Part of this filtrate was boiled and the other part left unheated. Two small holes slanting downward were bored, one in each side of a large lemon tree, and burettes holding about 50 cc. of the liquid were inserted into these holes and sealed. One burette contained a portion of the boiled filtrate just referred to, and the other unboiled filtrate. In each case the solution was taken up by the tree in a few hours. On November 5, 1912, a considerable quantity of gum was observed pushing up into the burette which had contained the unboiled filtrate. No gum was observed in the other burette. No further development had taken place in either case on November 30, 1912.

EXPERIMENT 2.—On April 16, 1913, filtrate from a large piece of lemon bark containing a lesion 35 by 8 cm. produced by inoculation with *Pythiacystis citrophthora* (Pl. 3, B) was obtained as described in the previous experiment. At the same time, a filtrate from a large piece of sound bark from a healthy tree was obtained in the same manner, as a control. The filtrate from the diseased bark was placed in a separatory funnel and was allowed to be absorbed by a young lemon tree (Pl. 8) through a small hole in the bark. About 100 cc. were taken up in five days. The filtrate from the sound bark was placed so as to be taken up by another lemon tree of the same size.

On May 6, 1913, thin gum was observed pushing up into the lower end of the funnel tube through which diseased filtrate had been introduced. Gum undoubtedly had begun to form internally long before this time. In about two weeks the gum (about 10 cc.) had accumulated in the lower enlarged portion to a height shown in Plate 8, after which the pressure resulted in this breaking through the rubber connections at the bottom. Very little gum was exuded after June 3, 1913, when the funnel was removed. The bark remained alive around the opening. During this entire time no gum appeared in the funnel through which the filtrate obtained from the sound bark had been introduced.

The results of these experiments indicate that there is a substance in the diseased bark which is capable of passing through a fine clay filter and of inducing gum formation. In the experiments, however, gum formation soon ceased without serious injury to the bark, such as is produced by the invasion of *Pythiacystis citrophthora*. It would seem, therefore, that the gumming is induced by some substance that is formed either by the fungus or by the interaction of the host and parasite. The fungus during its invasion of the host probably secretes a substance which stimulates either directly or indirectly the production of a gum-inducing enzyme by the host cells themselves. This substance doubtless also passes out into adjacent cells, and into the conducting tissue, and thus brings about gum formation at long distances from the invaded zone. This substance passing out from the invaded regions probably accounts for the large outer noninvaded gummy zones which so readily recover when the inciting cause, the parasitic fungus, is removed. This substance, capable of inducing gum formation, but not capable of bringing about the formation of lesions characteristic of *Pythiacystis* gummosis, appears to be destroyed by boiling and may, therefore, be an enzyme. Further investigation as to the real nature of this gum-inducing substance is needed.

## PARASITIC ORGANISMS AND GUM FORMATION

Although there are various agencies or diverse stimuli that contribute directly or indirectly to gum formation in Citrus, the more serious and progressive types of gummosis (in California at least) appear to be initiated by the invasion of certain parasitic fungi. With *Pythiacystis* gummosis, for sample, the bark and cambium region are first invaded and killed by the fungus which appears to advance most rapidly in the inner bark adjacent to the cambium. Soon after a small portion is killed by the initial invasion of the fungus, an influence is exerted bringing about gum formation in the xylem region surrounding the dead portion. After some time gum begins to form rapidly within the xylem for long distances vertically, but usually for only short distances laterally, from the invaded zone. The parasite, therefore, must stimulate such gum formation in an indirect manner, through setting into action some substance which passes out into the conducting system, and which moves probably in the sieve tubes as well as in the wood vessels. This gummy degeneration within the outer gummous zone usually does not kill the bark nor prevent its recovery, provided the parasite does not invade it later. A thin outer layer of wood, not penetrated nor killed deeply by the parasite, becomes infiltrated with gum, and occasionally gum pockets are formed within it but usually near the cambium.

The seriousness of excessive gum formation in itself has been overemphasized by some investigators because it was erroneously held that the death of the bark was the usual cause of gum formation instead of being the result of fungus invasion. Gum formation appears to diminish rather than to increase the seriousness of the diseases. As has been previously mentioned, the excessive gum formation in connection with *Pythiacystis* gummosis is frequently accompanied, or is soon followed, by a stoppage in the further advance of the invading parasite. It is highly probable that the infiltration of tissue by gum in the outer gummous zone tends to hinder the further advance of the mycelium into this tissue. In *Diplodia* gumming, Earle and Rogers (21) state that their observations have convinced them that gum flow in Citrus serves as a "protective device," and in plum wilt, Higgins (39) concluded that the gum served to hinder the advance of the organism by being formed in its path of advance. In the disease due to *Sclerotinia liberthana* on Citrus twigs, C. O. Smith (54) states that with the appearance of the gumming of the twig the further enlargement of the lesion is checked.

The gum in connection with *Pythiacystis* gummosis lesions, in addition to being a probable hindrance to the organism, serves in dry weather as a seal or covering to the wood beneath the killed bark, thus preventing the entrance of wood-destroying organisms and preventing excessive drying-out of the exposed wood. The gum being soluble in water, however, it is of little value for this purpose after the rains appear.

## BRIEF GENERALIZATION ON GUM FORMATION

The explanation of gum formation itself, which seems to fit in best with the known facts and results obtained with Citrus, is that a substance of an enzymatic nature may be brought into action through various instrumentalities, such as substances acting under the influence of invading organisms, introduced or induced chemical stimuli, etc. It appears to be evident that the enzyme immediately responsible for gum formation is

produced by the host tissue itself; otherwise it would be difficult to account for the gum formation induced by such a variety of chemical substances and agencies. In *Pythiacystis* and *Botrytis gummosis*, for example, there would seem to be two possible explanations of the manner in which the gum formation is brought about: (1) A substance secreted directly by the fungus sets in motion the gum-forming enzyme or (2) a substance resulting from the interaction of parasite and host furnishes the means by which the gum-forming enzyme is brought into action.

In case of the action of chemicals, such as corrosive sublimate and sulphuric acid, the substance might act also in either of two ways analogous to that given for the parasite, either directly in the same way as a secreted substance, or indirectly through a substance produced by the interaction of the plant tissues and the introduced chemical. The experiments with sterile filtrate from diseased and healthy bark previously described, taken in conjunction with the work of Floyd, Higgins, and others, appear to show that whatever may be the indirect factors in gum formation, the immediate cause or stimulus to its formation is a filterable substance of the nature of a heat-sensitive enzyme. It is evident that many important phases of the subject of gum formation in *Citrus* remain for further investigation.

#### SUMMARY

(1) A destructive form of *Citrus gummosis* attracted attention first in the Azores in 1834. A similar gum disease appeared in Italy as early as 1863; in Portugal, 1865; in Australia, 1867; in Spain, 1871; in United States of America, 1875; and in most other *Citrus* regions before the year 1890.

(2) *Pythiacystis gummosis*, the most widespread and destructive type of *Citrus gummosis* in California, is characterized by copious exudations of gum and dead patches of bark on the trunk and main roots. The gum may arise not only from the margin of the invaded area but also from a large contiguous, outer, noninvaded zone.

(3) It has been shown that the disease with all its usual symptoms may be readily transmitted to healthy trees by inoculation with bits of bark tissue cut from the advancing margins of killed regions of bark. It is not transmitted, however, by tissue from surrounding outer gummy zones or by killed tissue not recently invaded.

(4) Cultural tests have shown that live mycelium of *Pythiacystis citrophthora* Sm. and Sm., formerly known as the cause for lemon brown-rot, is present in this narrow band or fringe at the advancing edges of the killed region of bark (invaded zone) but is absent or dead elsewhere.

(5) Numerous inoculations with pure cultures of *Pythiacystis citrophthora* into healthy trees under various conditions have shown that this fungus is capable of inducing gummosis with all the characteristic symptoms of naturally occurring cases. The fungus has been reisolated from many lesions after it has remained in the bark from 1 to 11 months.

(6) Lemon fruits affected by brownrot due to *Pythiacystis citrophthora* have been shown to be capable of inducing the same type of gummosis as that produced by the fungus from gummosis lesions.

(7) The organism appeared to die out more readily and the lesions became self-limited more quickly after inoculation in branches or large roots than after inoculation in the trunk.

(8) A limited number of inoculation tests gave some indication that *Fusarium* sp., found commonly in connection with *Pythiacystis* gum-

mosis lesions, increased the severity of the disease when associated with *Pythiacystis citrophthora* but that *Fusarium* alone was not capable of initiating this gummosis.

(9) Observations and inoculation experiments both indicated that the order of resistance of species and varieties to *Pythiacystis citrophthora* from highest to lowest, is, sour orange (*Citrus aurantium* Linn.), trifoliolate orange (*Poncirus trifoliata* Raf.), rough lemon (a resistant variety of *Citrus limonia* Osbeck), pomelo (*Citrus grandis* Osbeck), sweet orange (*Citrus sinensis* Osbeck), and common lemon (*Citrus limonia* Osbeck). The first is almost immune, the last very susceptible.

(10) Inoculation of small roots of young trees indicated that common-lemon roots are somewhat susceptible but that sweet-orange, pomelo, and sour-orange roots are resistant.

(11) Mal di gomma due to *Phytophthora terrestris* Sherb. has been shown to be similar to certain phases of *Pythiacystis* gummosis, especially to the form the latter takes at the junction of the main roots and trunk of old orange trees in California.

(12) Inoculations with *Phytophthora terrestris* and with *Pythiacystis citrophthora*, under the same conditions, produced lesions which showed no characteristic differences.

(13) Experiments have shown that in cases where excess of moisture and other contributing conditions cannot be entirely avoided the disease may be prevented largely by applying Bordeaux mixture or other fungicides to the trunks.

(14) It has also been shown that the progress of the disease may be readily prevented by dissecting out the bark invaded by the causal organism and applying a fungicide. It was not found necessary to remove the bark in the outer gummosis zone, since this bark would finally recover after the advancing fungus had been removed.

(15) Botrytis gummosis of lemon trees is characterized in the early stages by a soft area of invaded bark killed to the wood, with exudation of gum on the trunk. Later this soft area becomes surrounded by a larger, firmer area in which only the outer layer of bark is killed, leaving a layer next to the cambium region alive. There is also, as in *Pythiacystis* gummosis, a noninfected outer gummosis zone from which copious gum exudation may take place.

(16) A strain of *Botrytis cinerea* Lk. has been found commonly associated with this type of gummosis and has been isolated from numerous lesions.

(17) Pure cultures of the fungus, as well as bits of the diseased bark, were found capable of inducing the disease when inoculated into cuts and other kinds of injuries on healthy lemon trees.

(18) Attempts made to induce gum formation by various kinds of wounds on lemon tree trunks invariably failed when these wounds were kept clean and free from contamination with injurious organisms or chemical substances.

(19) The experimental results show that the disease may be prevented by avoiding injuries to the bark and by using a fungicidal coating on its surface. The treatment found effective consists in cutting or scraping away the dead bark, leaving as much of the live bark as possible, and painting the treated area with a fungicide.

(20) *Sclerotinia libertiana* is occasionally found associated with rapid dying of bark and copious gum exudation on trunks and roots. The

bark is at first soft, later dries out into long shreds, and usually contains flat sclerotia within it.

(21) Inoculations with pure cultures have shown that this fungus may also kill the bark rapidly and bring about the results just mentioned on healthy lemon tree trunks.

(22) Inoculation experiments with a large number of other organisms showed that some of them were capable of inducing gum formation, but this effect was usually slight as compared to that of *Pythiacystis*, *Botrytis*, and *Sclerotinia*. Those which produced some gum exudation in cuts were *Penicillium roseum*, *Fusarium* sp., *Diplodia* sp., *Coryneum berynkii*, *Coprinus atramentarius*, *Alternaria* sp., and *Hypholoma* sp. The effect in killing of bark was insignificant, and no definite diseases resulted. Several other organisms produced no effect whatsoever either in inducing gum exudation or killing tissue.

(23) In Parts I and II of this paper the relation of certain fungi to definite types of diseases in which gum is a conspicuous feature has been discussed.

(24) Gum in Citrus is similar to gum arabic and cherry gum and appears to originate mainly in the xylem tissue by hydrolysis of the cellulose walls.

(25) Attempts to induce gum formation in lemon trees by various mechanical injuries, by obstructions placed in the conducting tissue, and by pressure on the bark, failed when the tissues experimented upon were free from parasitic organisms or unusual chemical stimuli.

(26) Injuries from certain insects have been observed to result in slight gum formation, which is probably due to secretions by the insects or to contaminating organisms.

(27) Experiments and observations indicate that burning and freezing are not important factors in inducing gum formation in Citrus but merely serve to open up the way for parasitic and wood-rotting fungi, which afterwards induce gumming.

(28) Partial desiccation appears to be merely a factor in the acceleration of the process of gum formation in Citrus and not a necessary condition to its initiation.

(29) Certain chemical substances are capable of inducing gum formation when injected into Citrus bark. It has not been possible, however, to reproduce all the typical symptoms of any of the gum diseases by chemical injections. The invasion of certain parasitic organisms appears to be the chief factor in initiating gum formation under natural conditions. These organisms may bring about, as do certain chemical substances, gum formation over considerable areas of bark surrounding the portions killed by either the organism or the chemical substance.

(30) Comparative experiments with boiled and unboiled filtrates from diseased and healthy tissue show that the diseased bark contains a substance capable of passing through a fine clay filter and of inducing gum formation when unboiled. When boiled, however, this capacity to stimulate gum is destroyed. This indicates the presence of a heat-sensitive enzyme.

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## PLATE 1

*Pythiacystis gummosis* on lemon trees, from inoculation with diseased bark tissue.

A.—Tree inoculated on February 27, 1912, the gum formation showing the development of disease on August 2, 1912.<sup>a</sup>

B.—Same tree, September 19, 1912, over 6 months after inoculation.

C.—Same tree about 1 year later, view at right angles to B. Only 5 cm. of live bark then prevented complete girdling.

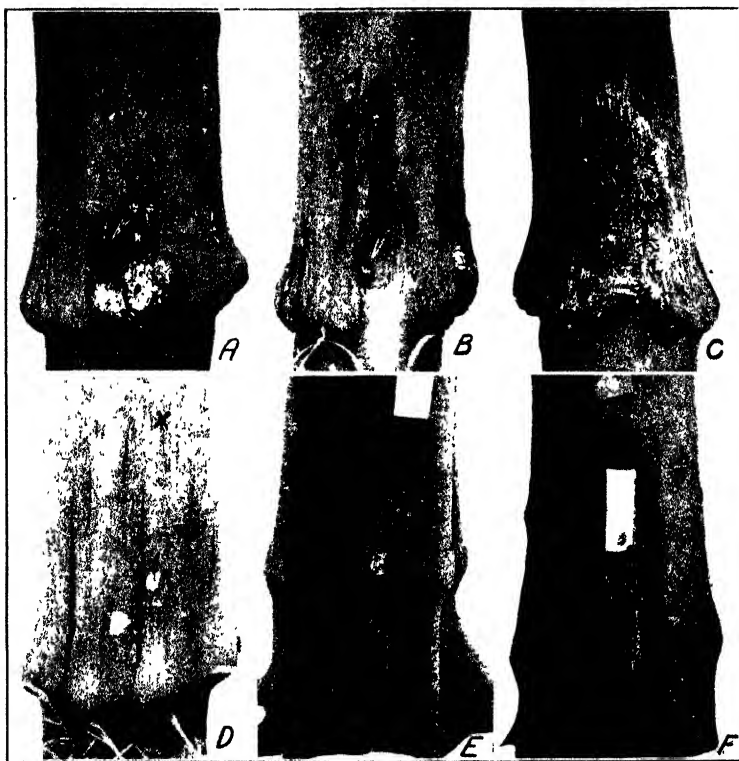
D.—Same view as A and B, on May 24, 1913, 15 months after inoculation. The gum first formed has been dissolved away by winter rains and the dead bark has dried and shrunk. Gum was exuding farther around, as in C, at this time.

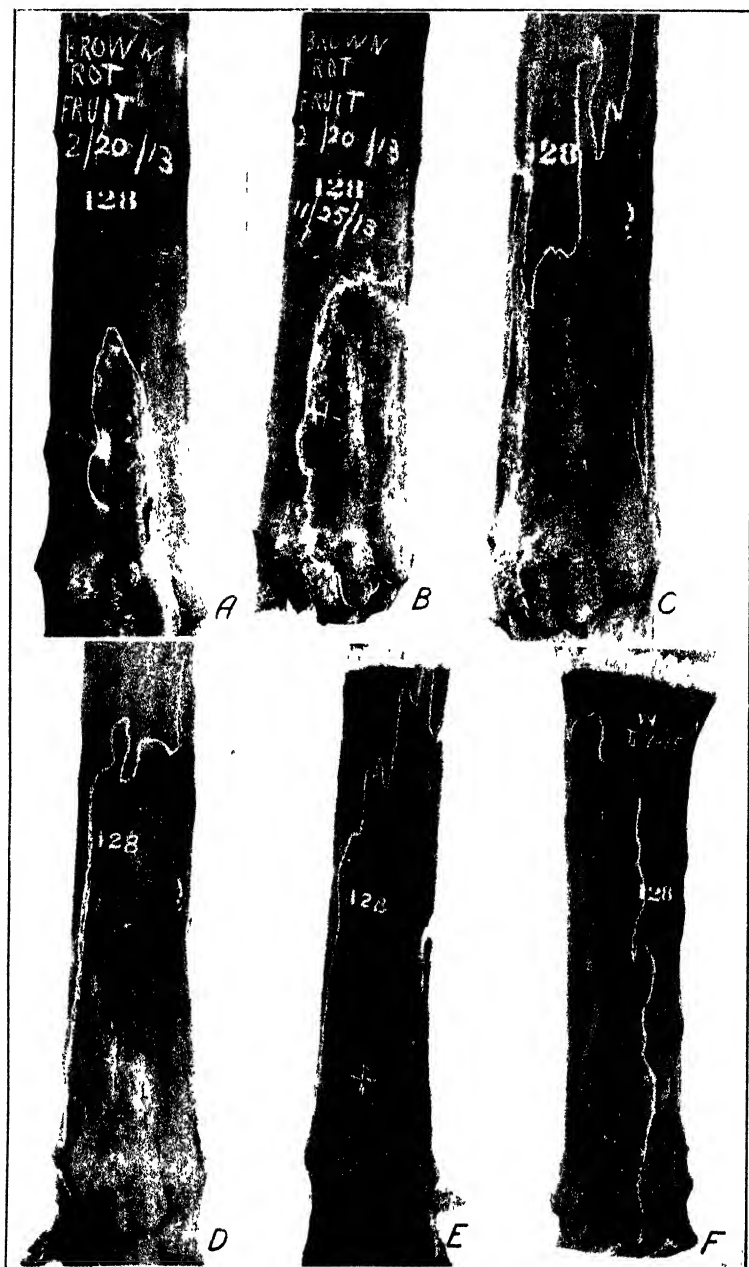
E.—Lemon tree inoculated with diseased bark tissue, September 21, 1912, showing excessive exudation of gum 2 months later.

F.—Lemon tree inoculated with diseased bark under a glass slide held with putty, September 21, 1912, showing gum formation 2 months later.

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<sup>a</sup> A majority of the trees in this same orchard had the same "overgrowths" at the union of stock and scion as observed in this illustration.





## PLATE 2

Pythiacystis gummosis on lemon tree from inoculation made on February 20, 1913, with diseased fruit tissue affected with Pythiacystis-rot.

A.—Extent of invaded bark and gum formation about 5 months after inoculation.

B.—Same tree about 7 months after inoculation, showing increase in the invaded area.

C.—Same tree about 13 months after inoculation, showing cracking of dead bark over invaded area and its large increase in size.

D.—Same tree 18 months after inoculation, showing a still larger area affected.

E, F.—Two views of the same tree, about 2 years after inoculation. Figure F taken on opposite side of tree from figure E.

### PLATE 3

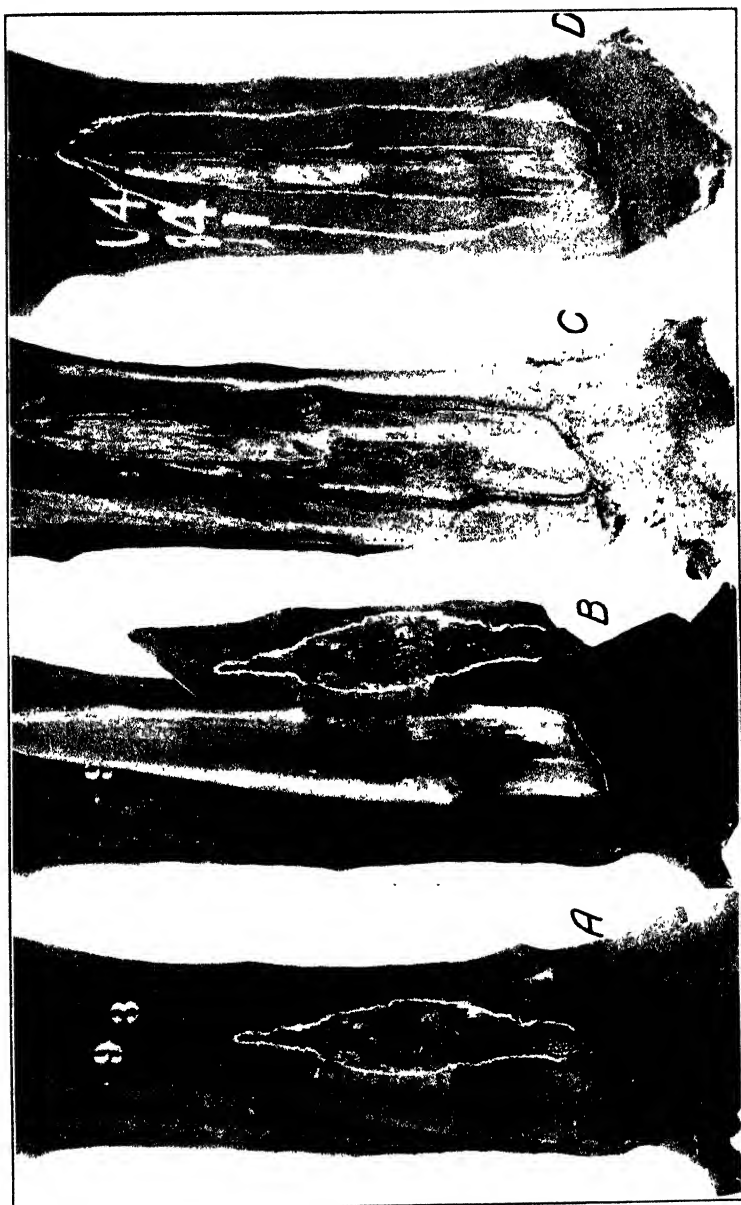
*Pythiacystis gummosis* on lemon tree from inoculation on November 23, 1912, with pure culture of *Pythiacystis citrophthora*.

A.—Extent of invaded portion (inside of chalk line) and gum formation on June 6, 1913. The fungus was isolated from several places near chalk line at this date.

B.—Same tree after bark was cut, showing invaded zone (black) and a part of the outer gummosis zone (shaded), which extended upward and downward under live bark.

C.—Same tree in September, 1914, showing new bark pushing in over wound.

D.—Same tree in June, 1920, showing increase in the new bark covering the edges of the original wound.





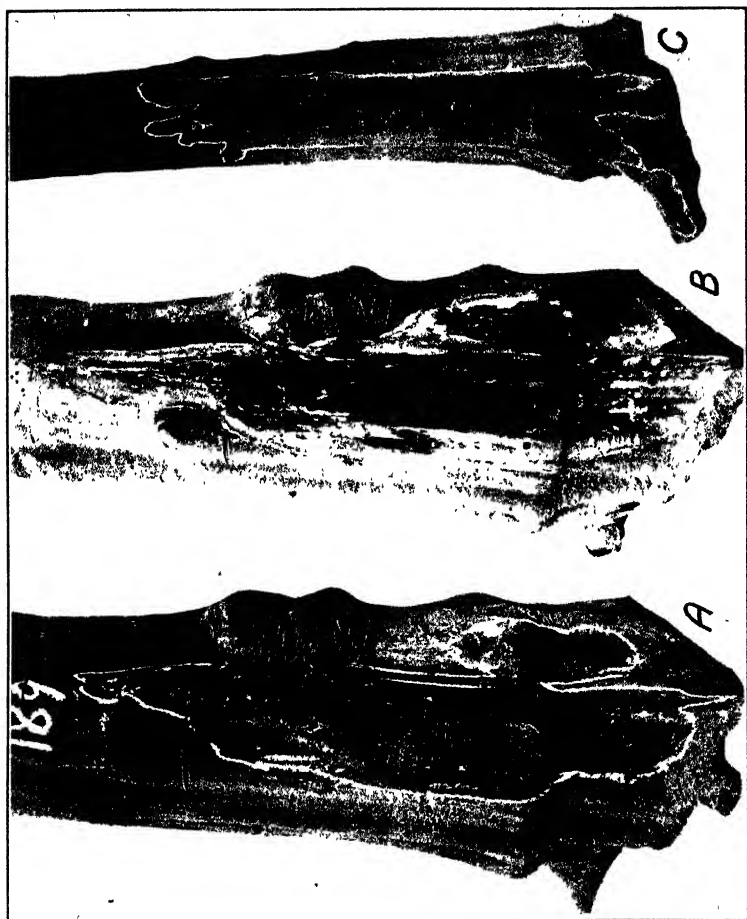


PLATE 4

*Pythiacystis gummosis* from inoculations made July 13, 1913, with pure cultures of *Pythiacystis citrophthora* on different parts of the same lemon tree.

A.—Extent of invaded zone and gumming from an inoculation on the sweet-orange stock. Photographed May 9, 1914. The fungus was isolated from upper portions near chalk line at this date, about 10 months after inoculation.

B.—Same, with bark removed, showing darkening at cambium region over invaded zone. Original inoculation was at the place marked +, from which the invasion of the fungus proceeded mostly upward, over the "bud union" (swollen ring) into the lemon bark.

C.—Opposite side of same tree on same date, showing portions invaded (chalk line) as result of two inoculations, one on lemon bark above and one on a large crown root below the bud union.

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## PLATE 5

*Pythiacystis* gummosis which was apparently increased in severity by inoculation with both *Fusarium* sp. and *Pythiacystis citrophthora* February 22, 1913, showing progress of disease over a period of about 3 years. This tree was finally girdled and killed by disease starting from one inoculated cut.

A.—Exuded gum and extent of invaded zone (chalk line) July 28, 1913, 5 months after inoculation. Arrow indicates point of inoculation.

B.—Extent of the lesion on same tree November 25, 1913, 9 months after inoculation. The infection has extended downward nearly as far as upward.

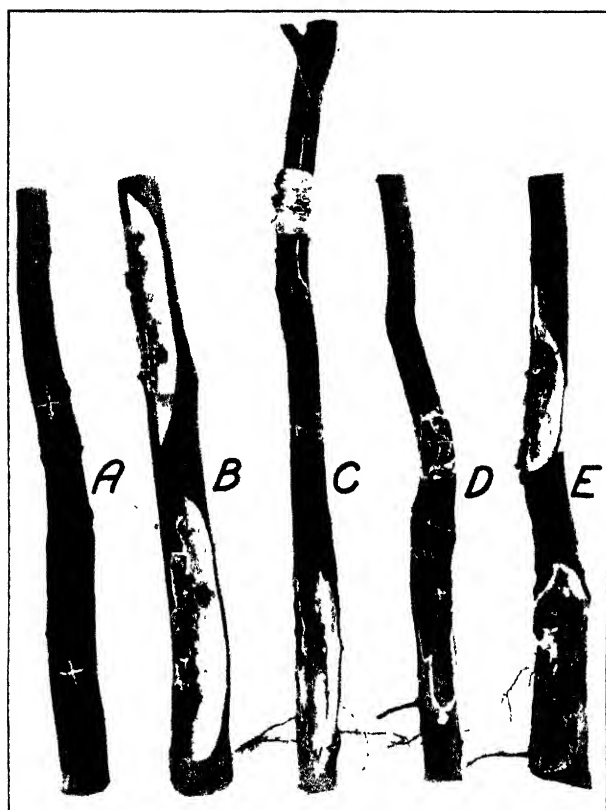
C.—Extent of lesion and cracking of bark on April 9, 1914, more than 13 months after inoculation. The gum of figures A and B has been dissolved by rains.

D.—Large quantity of exuded gum and a great enlargement of invaded zone and cracking of bark, on September 4, 1914, about 17 months after inoculation.

E.—Further cracking of dead bark on February 6, 1915, nearly 2 years after inoculation. The gum of figure D has been dissolved by further rains.

F.—Trunk on opposite side from figure E, on same date. Only a small strip of live bark here remains, which was later invaded, this resulting in the death of the tree.





## PLATE 6

Results of inoculation with pure cultures of different strains of *Phytophthora terrestris* and with *Pythiacystis citrophthora* on young lemon trees (orange stock at lower inoculated cut). Inoculated April 20, 1914, photographed August 26, 1914. (See Table XI.)

- A.—Control cuts without inoculum.
- B.—Inoculated with *Phytophthora terrestris* from Isle of Pines. Bark cut away to show extent of lesions.
- C.—Inoculated with *Pythiacystis citrophthora* from California.
- D.—Inoculated with *Phytophthora terrestris* from Cuba.
- E.—Inoculated with *Phytophthora terrestris* from Florida.

# PLATE 7

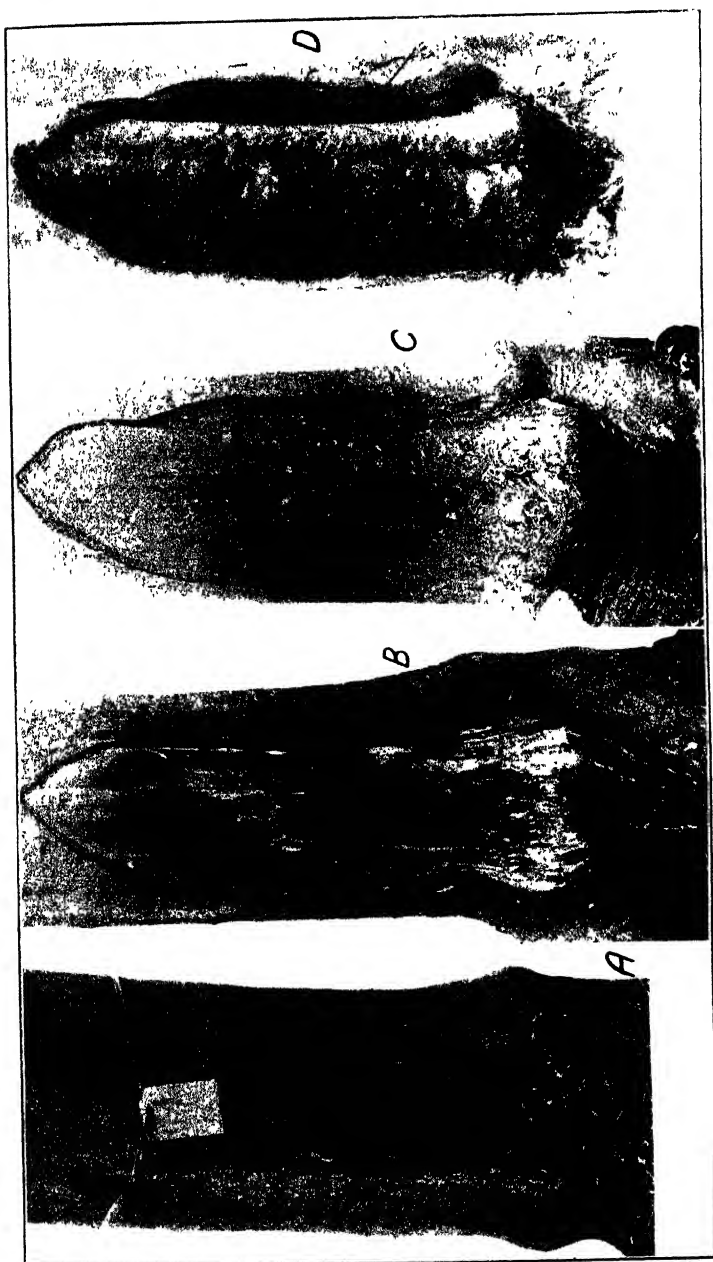
Method of cutting away diseased bark in treatment of a severe case of *Pythiacystis* gummosis.

A.—Result of inoculation with diseased bark containing *Pythiacystis citrophthora* on November 16, 1912. Photographed September 3, 1913. Invaded area 21.5 by 7.5 cm.

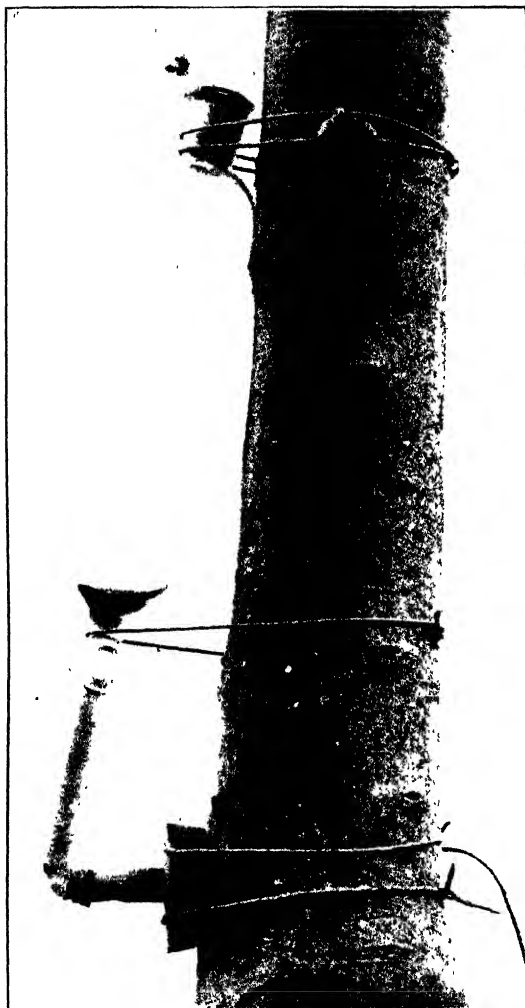
B.—Bark cut away September 3, 1913, on a similar tree in treatment.

C.—Same tree after painting with Bordeaux paste.

D.—Same tree on March 17, 1916, showing growth at edges of wound and asphalt paint on exposed wood.







**PLATE 8**

**Gum pushed up into bottom of funnel from which sterile, unheated, and filtered extract from bark affected with *Pythiacystis gummosis* had gone into the tree about 4 weeks before.**



# OCCURRENCE AND SIGNIFICANCE OF PHLOEM NECROSIS IN THE IRISH POTATO<sup>1</sup>

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## INTRODUCTION

In a recent publication Esmarch (5)<sup>2</sup> makes the following generalization in regard to the condition of the phloem:

Necrosis of the phloem is found in both normal and diseased plants and is always present in mature organs; if necrosis is observed during the early life of the plant, it is an indication of premature ripening.

In support of this theory, which is based on his own observations and supplemented by the data of other writers (7), Esmarch compares the changes in the phloem of the potato to those taking place in the secondary phloem of woody plants. Here, with the disintegration of the nucleus and the formation of callus deposits over the plates, the sieve tubes become inactive. The empty elements, deprived of their turgor, collapse and are crushed by the surrounding tissues. As obliteration progresses the old phloem often becomes so changed structurally and chemically as no longer to resemble its former state. However, while the obliteration of the phloem in woody plants, as has been shown by numerous investigators (3), is comprehensible on structural and physiological grounds, similar changes in the phloem tissue of herbaceous plants may not be expected, nor is their frequent occurrence reported in literature. Indeed, our present knowledge is restricted to two short notes by Boodle and Schuman. Boodle (4) observed in *Helianthus annuus* the occurrence of sieve tubes and companion cells whose walls were lignified and whose content gave reactions resembling those of lignin. Schuman (8) mentions that sclerosis of the phloem takes place in some few woody Composites and gives *Scorsonera hispanica* and *Aster thyrsiflorus* as examples. A systematic study of the mature phloem, carried on recently in the botanical laboratory of Cornell University, indicates clearly that most herbaceous plants do not exhibit any changes in the phloem upon maturation; where changes do take place, the phloem elements become lignified without obliteration.

A consideration of anatomical changes in diseased plants has often been fruitful in providing diagnostic symptoms for the identification of plant diseases, provided, however, that the ontogeny of the normal parts was fully understood. It is imperative, indeed, to distinguish clearly between normally expected tissue changes, such as take place

<sup>1</sup> Accepted for publication July 11, 1922.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 245.

in the phloem of woody plants, and induced abnormal states, the latter alone being truly pathological. The apparent discrepancies noted by different investigators may, and usually do, become intelligible if we consider all pathological changes and trace the causes of the various types of necrotic conditions, if possible, to their sources.

A classical example of such discrepancies is afforded by the researches on the pathological anatomy of the potato in connection with leafroll. To be sure, the views of the different writers are substantiated by observations, but since statistical data are incomplete at best, only a purposeful and systematic investigation of the problem can provide adequate data, even though factors which govern plant growth be not altogether neglected in the analysis of results.

To arrive at some definite basis as to what constitutes a healthy potato plant from the viewpoint of the anatomist, and under what conditions the phloem will remain normal, an investigation of a large number of plants of both cultivated varieties and indigenous South American forms was carried on. Such studies have been in progress since the summer of 1916, and the data obtained have been embodied in part in earlier publications (1, 2) and have been extended and partly modified in the present paper. Further work on the pathological anatomy of potato diseases will doubtless bring forth new interesting facts and greatly aid in analyzing these complicated potato disorders of which the cause is only vaguely understood.

#### THE NORMAL PHLOEM

The vascular tissue of the potato plant shows a bicollateral arrangement of its elements, a condition most clearly seen in the larger stem bundles (fig. 1). The primary phloem, external to the cambium, is made up of small groups of cells more or less continuous; the groups constituting the inner phloem are very variable in size and scattered. Groups of primary phloem appear also in the interfascicular region, where they may be seen on both sides of a well-developed cambium. Through branching and anastomoses the individual groups in each region communicate with one another, while through branch and leaf gaps a similar connection is effected between the inner and outer phloem.

During the early vegetative development only groups of primary phloem are seen in a cross-sectional area of the stem. However, when tuber formation is under way, the cambium gives rise to a broad band of phloem (fig. 2). The amount of this secondary phloem varies with the location. In the nodal region the amount exceeds that found in the internode, and in a given cross-sectional area, the larger amount is always found on the face of the larger stem bundles.

While secondary phloem elements become differentiated and take part in the translocation processes, the primary phloem groups remain active until the plant is mature. The walls of the cells thicken slightly, and occasionally callus deposits cover the plates of the sieve tubes. Otherwise, there are no noticeable changes, either structural or chemical, characteristic of the phloem of the mature plant.

Local necrotic changes in the parenchymatous tissue, however, may be observed in any potato plant. The diseased areas are usually restricted and do not extend vertically for any appreciable distance. In the distal stem region, especially the node, such small pathological areas are of frequent occurrence, but they usually disappear in the maturing

organs. The first appearance of such a diseased area is exhibited by a swelling in the cell wall, often accompanied by discoloration. At a later stage, browning of the walls is noted, while at the same time the

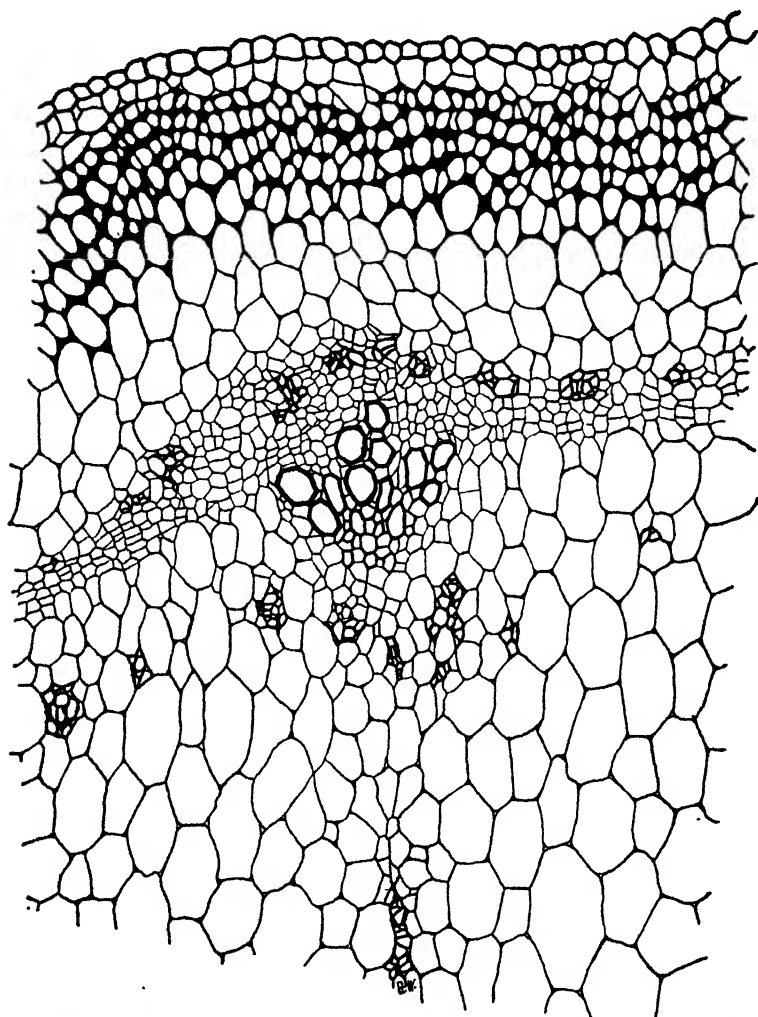


FIG. 1.—Stem section of young normal potato plant, showing general anatomical arrangement of vascular and fundamental tissues.

lumen is partly or entirely filled with a gummy deposit which is homogeneous or granular in nature. The parenchyma cells in the region where phloem fibers are differentiating show commonly an abnormal swelling, local in nature or extending laterally to include the walls of the peripheral phloem cells.

Any theory which could be postulated to explain the pathological disturbances in seemingly healthy individuals would naturally contain much of the hypothetical and be antecedent to our present knowledge of physiological phenomena. Temporary and local changes in the metabolism may cause the production of substances toxic to young and delicate cells. Microorganisms, which have occasionally been isolated from normal plants, could, under certain conditions, cause physiological

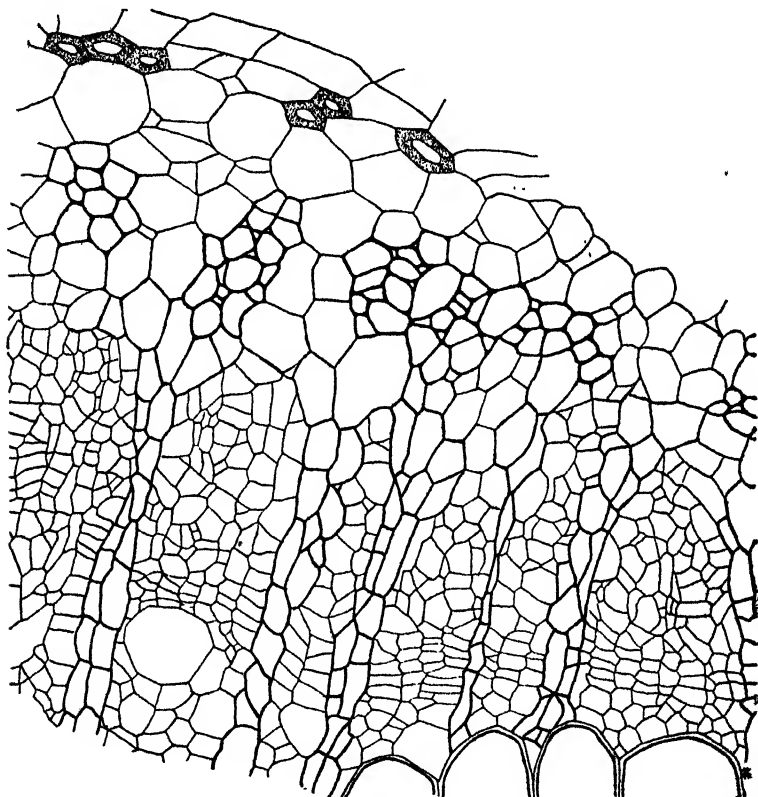


FIG. 2.—Mature normal stem section. Secondary phloem is well developed and by far exceeds in amount the primary tissue.

disturbances with accompanying cytological changes. Mechanical injuries may directly or indirectly produce similar effects. However, in the latter case it should be possible to trace the origin of the diseased area and to determine whether the cause was external or internal, and this has often been accomplished.

#### INFLUENCE OF ENVIRONMENT

An insight into the effect of environmental factors on the anatomical structure was obtained by studying a number of potato varieties grown at the high altitude station at Fort Lewis in Colorado. By taking ad-

vantage of the natural great diversity of ecological conditions, almost any desired combination of soil, moisture, and climatic factors could be had. Only where the natural agencies were inadequate or where a special combination of factors seemed advantageous was artificial control of these factors resorted to. Material for the study of the effect of progressively increasing water supply was taken from a field a part of which had become inundated by seepage from an adjoining mesa.

Plants suffering from an oversupply of water showed anatomical changes resembling those observed in foot diseases. The primary xylem and parts of the secondary elements of the wood were discolored and the lumen was filled with a brown, gummy deposit. The phloem part of the vascular tissue was commonly unaffected, and in cases where necrosis of the phloem was observed the material came from doubtful stock. The diseased phloem groups, which were occasionally observed in such material, did not take the lignin stain but showed the typical brownish discoloration which is characteristic of necrosis in general.

A greatly reduced water supply, or alternate wetting and drying, produced a denser and more strongly lignified wood. It neither inhibited nor accelerated the production of pathological changes.

The effect of shading was found to be closely bound up with the photosynthetic activity of the plant. Plants which grew to maturity in the shade of the river bottom vegetation were rank and slender. The xylem was greatly reduced and lignification of the cells less pronounced. The phloem remained normal throughout the life of the plants.

Thus, qualitative changes in the phloem, as a result of extremes of environmental conditions, are uncommon. When present, the diseased groups are localized and are connected with or are under the influence of necrotic areas in adjacent tissues. The pathological changes in the phloem tissue consists in a browning, rarely a typical lignification of the cell walls.

These observations, together with the fact that obliteration of the phloem, as a natural phenomenon in normal herbaceous plants, is discredited on theoretical grounds and disproved by extensive systematic studies, emphasized once more the fact that generalizations based on inadequate observations may lead us astray in the interpretation of true pathological changes.

#### NECROSIS FOLLOWING EXTERNAL INJURIES

By far the most common source of internal stem lesions is external injury produced by insects. The ensuing cellular changes may either constitute a direct response to the mechanical injury or be the result of irritations causing abnormal cell division and necrotic changes in the affected areas.

The lesions become externally visible as abnormal swellings of the size of small pustules barely discernible to the naked eye (Pl. 1, A) or they are of the nature of large intumescences which may be seen in various stages of development. The oldest swellings are conspicuous by their brownish color and the fissured surface of the epidermis. The location of the lesions is the apical stem region and the young petiole. However, swellings may be found in any region regardless of the age and relative development of the organ. Concomitant with the formation of these intumescences is a change in the morphological development of the parts above the points of injury. The leaves are deformed and discolored. The leaflets fold along the midrib or roll in tubular fashion. The inter-



nodes are shortened and often appear almost telescoped. Withal, the morphological changes are of a type akin to the symptoms of leafroll and foot diseases.

An examination of a section through a swollen area discloses to the naked eye a dark discoloration of the tissues. A microscopic study shows further that both vascular and cortical tissues are affected and that aside from necrotic changes there are regions of abnormal cellular activity. Sections through the advancing margin of a young pustule show that the first evidence of pathological changes is found in the cambium region. The xylem has matured irregularly, giving the peripheral region of the tissue a jagged appearance. In places one may observe mature groups of xylem embedded in parenchymatous tissue and completely separated from the vascular ring. In the more central part of the swelling the tissues appear completely disorganized. In cortex and pith are small groups of brown cells surrounded by concentric rings of thin-walled periderm cells. This latter type of necrosis is, however, frequently met with in normal plants, or is found in connection with other injuries.

The phloem tissue, in the affected areas, shows the same pathological condition observed in general. The cell wall and content show a brownish discoloration, and in extreme cases entire cells or cell complexes may be obliterated.

#### NECROSIS IN CONNECTION WITH STEM STREAK

Potato tubers of the variety Irish Cobbler which were grown in the greenhouse developed extensive stem lesions on both young and maturing shoots. The lesions may best be referred to as "streak," the affected areas being elongated and brownish in color. Their advance along the stem is acropetal. In the nodal region the browning is especially pronounced and extends into the lower part of the petiole.

The lesions are the result of necrotic changes in the cells of the collenchyma and adjacent tissues. The hypodermis is often still green while the collenchyma cells underneath are brown and in a state of obliteration. Soon, however, being cut off from the water supply, both epidermis and hypodermis die and the walls take on the same brown color as the cells underneath. In advanced lesions the necrotic areas extend through cortex and vascular tissue into the pith. In the nodal region the entire pith is reduced to a cavity lined with obliterated and discolored parenchyma cells. If the epidermis from an advanced lesion is removed and cleared in chloral hydrate, the microscopic examination shows scattered through the homogeneous mass of brown cells small areas of dark-colored cells. These areas usually cluster around a stomate and extend centripetally into the pith.

The behavior of the phloem cells is of special interest in this connection. Severe necrosis may be observed in both inner and outer phloem (Pl. 2). The cells are brownish in color, the lumen distinct or partly closed by the pressure of the surrounding tissue. Characteristic intercellular spaces are also formed, and progressive lignification of the type diagnostic of leafroll is not uncommon. The necrotic phloem groups, however, seem to be restricted and limited by the extent of the external symptoms, there being no regularity either in their vertical or lateral distribution. It still remains to be shown whether this disease is definitely connected with leafroll, and, if in part distinct, whether certain aspects of the disease are not the result of secondary infection by the leafroll virus.

## TRUE PHLOEM NECROSIS AND THE LEAFROLL PROBLEM

In the microscopic examination of potato vines one observes, occasionally, pathological changes associated with certain types of diseased plants which by virtue of their peculiar external appearance are grouped under the collective name of leafroll. Quanjer (6), while investigating the leafroll disease, noticed this correlation, and as the result of subsequent studies arrived at the following conclusion:

The lignification of the phloem is a dependable diagnostic symptom for the identification of leafroll, and the physiological disturbance, occasioned by the destruction of the conducting system for plastic materials, accounts for the change in the morphological structure and for the reduction in yield.

Stem sections of a typical leafroll plant exhibit, as a diagnostic internal symptom, a necrosis and lignification of the phloem groups. In case of severe external symptoms the diseased groups pervade the entire plant with the occasional exception of the underground organs. The distal stem region is commonly affected, and in nearly every instance the necrotic changes are of an extreme type. The basal stem region always shows necrotic changes when external symptoms become evident while the plant is still young. As a rule necrosis of the phloem in the lower stem means general necrosis of the plant throughout its extent, but the symptoms may decrease toward the distal end or disappear altogether. At any given height of the stem the node is typically more severely affected than is the internode. This condition is especially observed in the initial stages of the disease, but during subsequent development either region may be equally affected.

In petiole and midrib necrotic phloem groups may appear at a much later period, but the extent of necrosis is as a rule correlated with the severity of the rolling and the discoloration of the foliage. In the young leaf most advanced necrosis is observed in the middle part of the petiole probably because this part of the leaf is ontogenetically the oldest. In mature leaves, however, the midrib is often the organ which is most severely affected.

In the underground organs of diseased plants the phloem strands are usually normal, but in severe cases both stolons and tubers will show evidence of necrosis.

The lateral distribution of phloem necrosis is also subject to a great deal of variation. This is true for the phloem groups of a certain region, but the difference becomes even more apparent when outer and inner phloem are compared. In a given cross sectional area one may observe phloem groups in various stages of degeneration. Entire groups may be affected in whole or in part. Often one notices perfectly healthy groups side by side with diseased ones, which seems the more remarkable if one recalls how closely the groups are connected with each other through branching and anastomoses. In the apical stem region the first stages of necrosis are found in the external phloem and only later in both regions. In the basal stem either region is found to be diseased, but often the inner phloem alone is completely destroyed while the outer phloem is altogether normal or shows, at the most, only initial stages of disorganization.

In petiole and midrib, necrosis is primarily restricted to the outer phloem. In advanced stages the bundles flanking the corners of the large lateral bundles are necrotic, and occasionally all the inner phloem is also diseased.

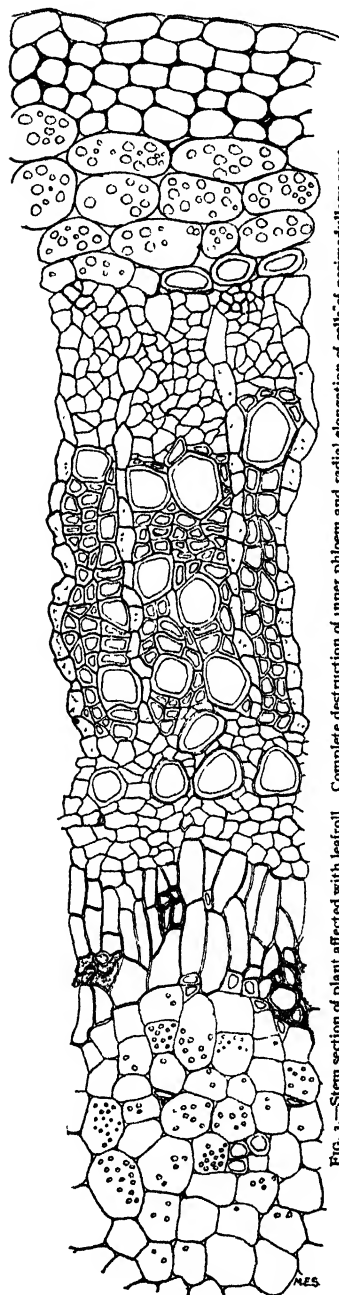


Fig. 3.—Stem section of plant affected with leafroll. Complete destruction of inner phloem and radial elongation of cells of perimedullary zone.

The progressive lignification of the phloem tissue can best be studied in sections taken through the distal stem region. Here one usually finds a complete series of changes from the first cell response to complete disorganization of the elements. Before there is any detectable evidence of lignification the development of the vascular tissue shows a deviation from its normal course, which is indicated by a more irregular maturing of the xylem than is found in healthy plants (Pl. 1, B). If such a section is stained with phloroglucin and hydrochloric acid, one notices upon close observation that the phloem cells centrifugal to the depression in the cambium show a slight amount of lignification in parts of the wall.

The cells of the pericycle in this region differ also from the normal type in having a greater radial diameter. As lignification progresses the entire cell wall or entire groups of cells become affected, while at the same time the radial elongation of the pericyclic cells of the outer phloem and the parenchyma of the perimedullary zone of the inner region becomes more pronounced (Pl. 3). The cells which first show lignification are commonly found adjacent to the fibers, but now and again lignification in a phloem group may start at the center and extend in a centrifugal direction.

Prior to lignification of the phloem, to be tested microchemically, a swelling of the walls of the diseased cells takes place. Ferrous sulphate and potassium ferrocyanid at this stage impart a deep blue color to the walls, indicating the presence of large quantities of pectic substances. The swelling of the walls extends from the region of the fibers centrifugally (Pl. 4, B). Gradually the cells lignify and progressively cells and groups of cells are withdrawn from active participation in conduction. In severe cases most or all of the primary phloem becomes destroyed (fig. 3; Pl. 5). In the initial stages of lignification of certain phloem groups the primary walls of adjacent cells swell and separate. The intercellular space

thus formed becomes filled with a brown deposit, which at a certain stage takes the lignin stain. Following the swelling of the wall there is often a disintegration of the wall substance. The swollen wall appears lamellate (Pl. 4, A) and between the lamellae spaces are formed which are filled with a gummy substance. The cell content also degenerates and disappears in part or becomes transformed into a substance giving reactions similar to the interstitial substance described above. Following the death of the cell and the subsequent loss of turgor, the phloem elements collapse, unless rapid lignification lends sufficient rigidity to the walls and prevents their being crushed by the surrounding tissues.

### CONCLUSIONS

The phloem of the potato, like that of the majority of herbaceous plants, remains normal throughout the vegetative period and up to late maturity of the plant. Although it is not affected by extremes in environmental conditions, it nevertheless undergoes pathological changes under the effect of certain metabolic disturbances such as probably exist in the leafroll disease. These changes consist in lignification or obliteration of cell wall and content. However, while obliteration of the phloem is always observed in connection with leafroll, it is also an accompanying phenomenon in other diseases. It is not so much its mere presence as its universality in distribution, coupled with the absence of necrosis in other tissues, which gives it a real diagnostic value.

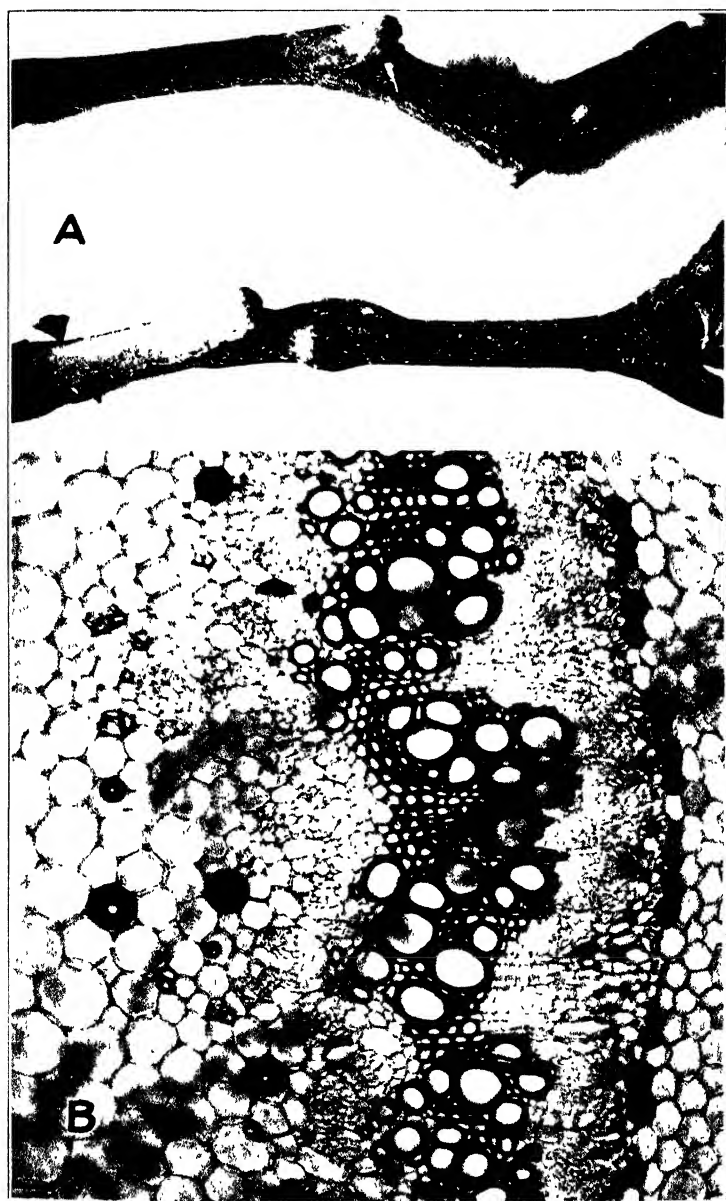
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PLATE 1

A.—Distal stem region. The swellings are due to insect injuries, they show as internal symptoms necrosis of vascular and cortical tissue

B.—Hand section of potato stem affected with leafroll. The xylem is maturing very irregularly, which gives the cambium zone a jagged appearance.



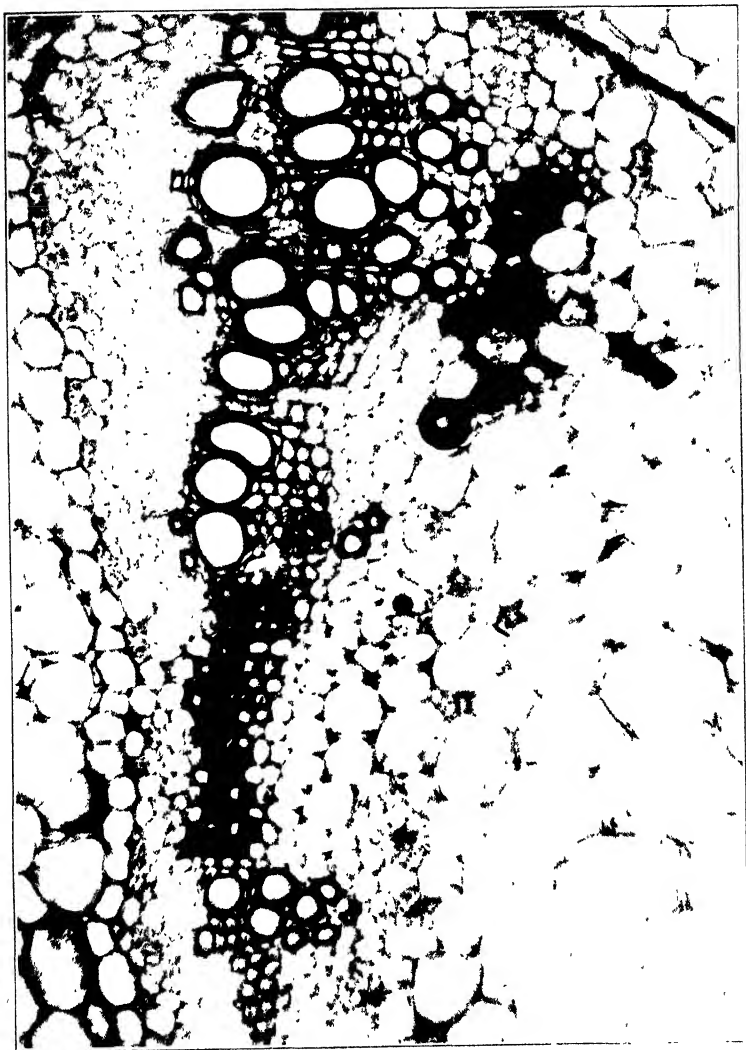


PLATE 2

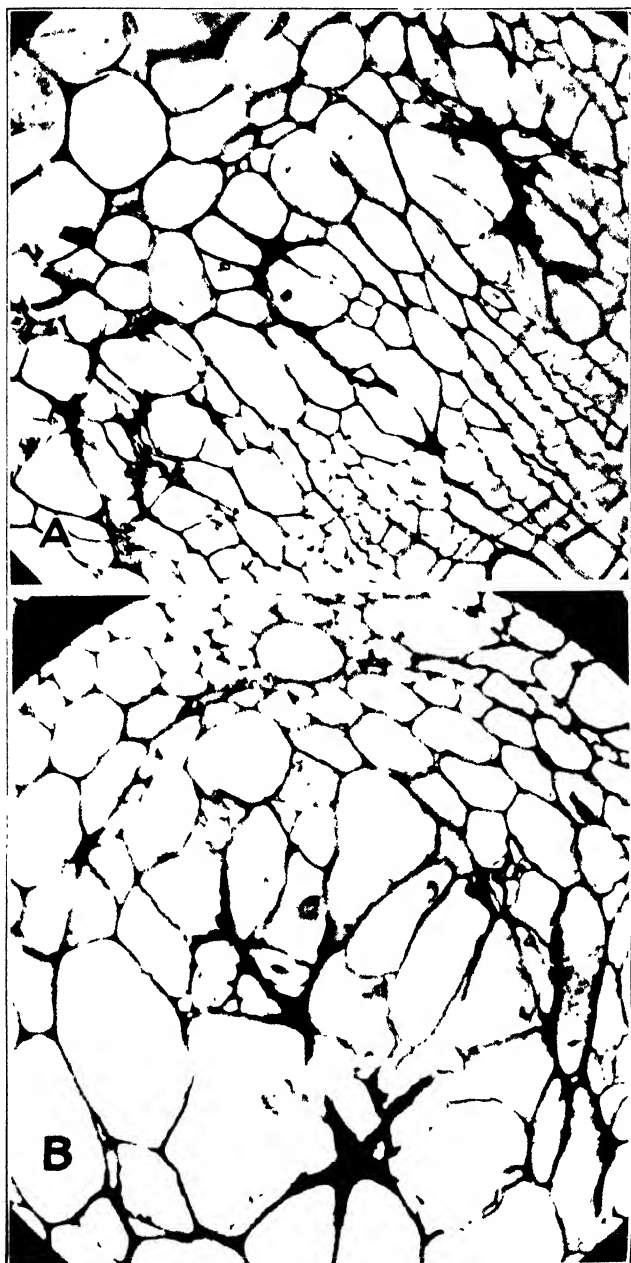
Hand section of potato stem affected with "stemstreak." Both xylem and phloem show necrosis. The necrosis of the phloem, however, is not a typical lignification.

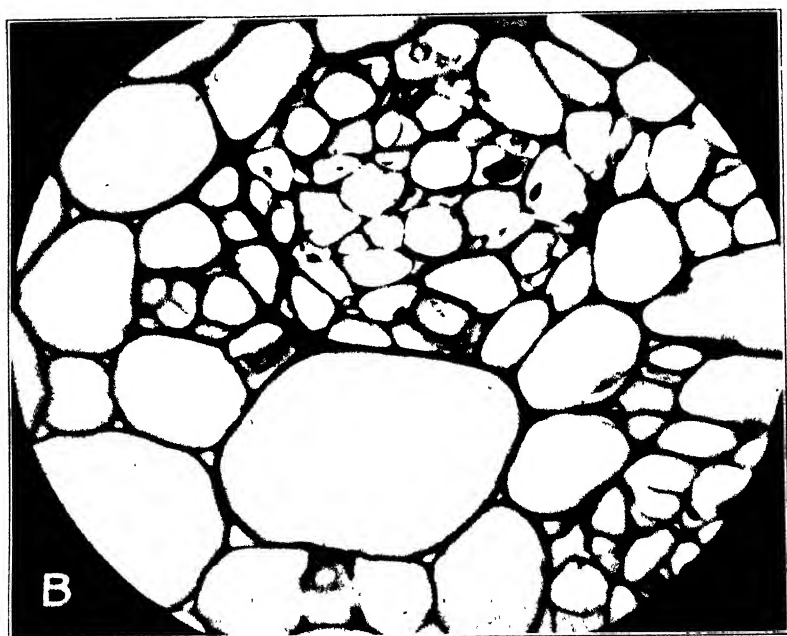
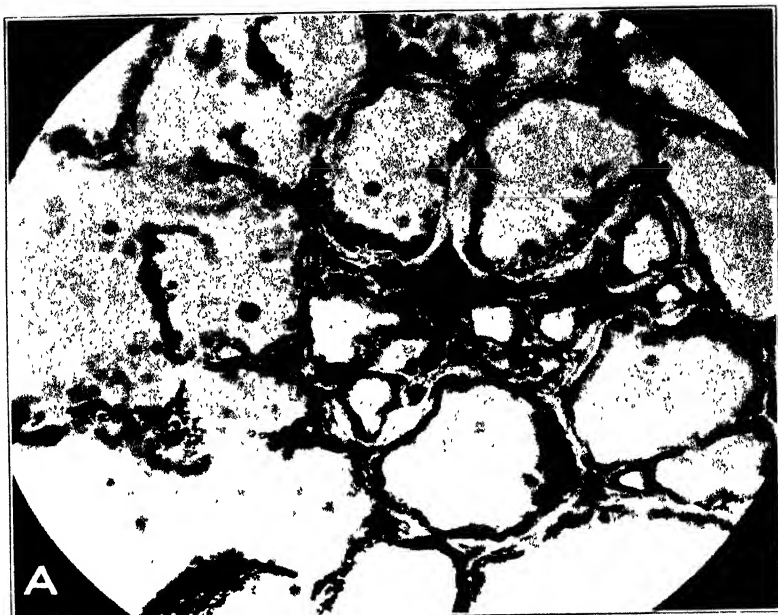
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PLATE 3

A.—Advanced necrosis of outer phloem in leafroll stem.  
B.—Advanced necrosis of inner phloem.





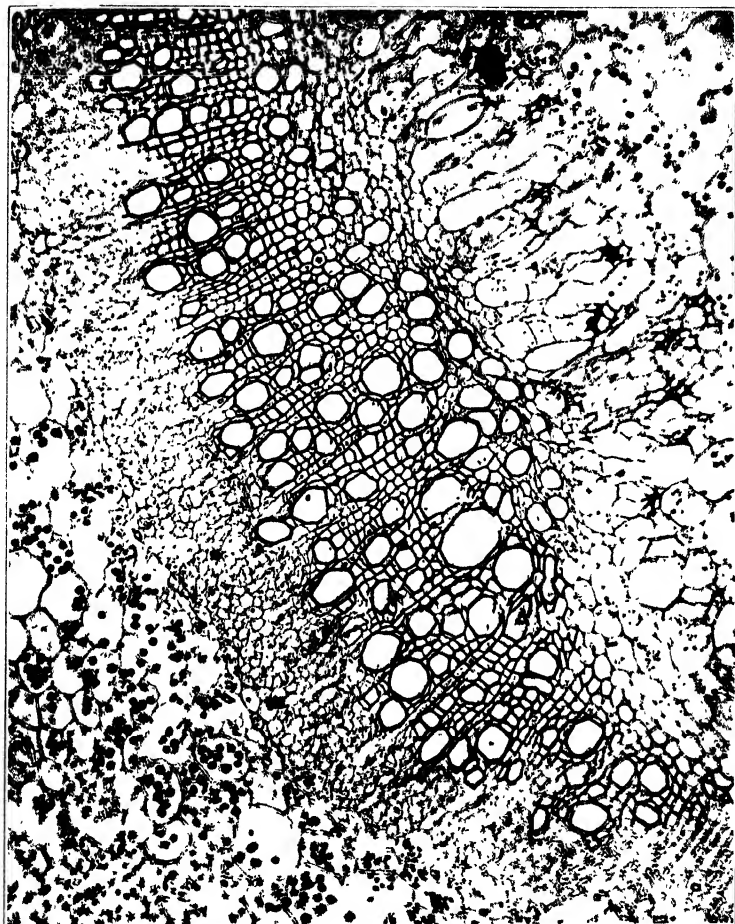
#### PLATE 4

A.—Enlarged view of inner phloem group, showing the minute pathological changes in the structure of the cell wall. (Gold chlorid stain.)

B.—Inner phloem group of potato stem, showing beginning stages of necrosis. Note the swelling of the walls of certain of the cells in the region of the fibers.

#### PLATE 5

Section through a large stem bundle of a mature stem affected with leafroll. The inner phloem is completely destroyed while the outer phloem, on the other hand, is normal.





# CULTIVATED AND WILD HOSTS OF SUGAR-CANE OR GRASS MOSAIC<sup>1</sup>

By E. W. BRANDES, *Pathologist*, and PETER J. KLAPHAAR, *Assistant Pathologist, Office of Sugar-Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## PURPOSE OF THE INVESTIGATION

Since 1919, when it was demonstrated by the senior writer that grasses other than sugar cane are susceptible to the so-called sugar-cane mosaic (3, 4, 5),<sup>2</sup> a large number of experiments have been performed to determine whether grasses in general are susceptible. It was especially important to learn whether the forage and field crops of the South are affected and, if so, to what extent damage is caused. On account of the comparatively recent introduction of the disease on sugar cane into this country, it was expected that crops in the vicinity of diseased sugar cane would be more likely to be affected. Accordingly these crops were closely scrutinized for symptoms similar to those on sugar cane, and when suspected cases were observed the plants were tested experimentally at Washington. Other closely related cultivated plants were tested, whether or not they showed signs of natural infection in the fields.

Another class of grasses, namely, the weeds or wild grasses found in sugar-cane fields, was held under observation, and any species which showed signs of disease were similarly tested. The importance of having exact knowledge of the susceptibility of wild grasses to grass mosaic lies in the fact that the disease may be carried over winter in perennial grasses and may reinfect the susceptible annual grass crops in the spring.

The rôle of wild perennial hosts in preserving the virus of mosaic through the winter has not been fully established, but they must be regarded as a potential source of danger, and our studies are justified on these grounds. In the South, even the annual grasses may be found in the growing state during the whole year. Sugar cane itself is a perennial, and the virus of mosaic is known to survive the winter in the stubble, as the ratoon plants invariably come up diseased in the spring if the plants of the previous year were infected. The attempt has been made in some regions to eradicate the disease by destroying all infected cane stools or by plowing out whole fields and planting to other crops for a year or more. The labor of course would be wasted if the disease were perpetuated in some unobserved wild perennial host. This method of eradication has been successful, however, in at least one sugar-cane region, where the disease was early observed and prompt and vigorous measures were taken to stamp it out. This region is the peninsular section of Florida where the disease no longer exists to our knowledge.

More than 40 grasses have been tested experimentally, either by artificial inoculation or by means of insect inoculation or both.

<sup>1</sup> Accepted for publication Dec. 1, 1921.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 262.



## EXPERIMENTAL INOCULATIONS

As a result of these experiments it has been conclusively proved that at least 13 species of grasses are subject to attack by the identical virus causing sugar-cane mosaic. Undoubtedly there are many more, but with the limited space at the disposal of the writers it was impossible to carry on the work on a larger scale. Species which have thus far given negative results may in the future prove to be susceptible. Many factors have entered which no doubt have diminished the chances for infection following these inoculations. Optimum growing conditions for many of the grasses do not prevail at Washington. Sometimes whole series of inoculations including known susceptible control plants failed, owing to unknown causes. Insect carriers became parasitized by other insects or died as a result of unfavorable conditions in the greenhouse, and they were not available or were not in a normal state when the plants were in the best condition for inoculation. In other words, the positive results attained must naturally carry more weight than the negative results, and in listing grasses as not susceptible it is merely meant that they did not become infected in these experiments.

Though it is possible that there is more than one mosaic disease affecting grasses, and there is some reason for believing this to be so, this paper is concerned with one only, the well-known type which has recently proved so destructive to sugar cane in Porto Rico and the southern United States.

The plants tested and found susceptible are shown in the following list:

## Cultivated crops:

## Perennial—

Sugar cane.....*Saccharum officinarum*.

## Annuals—

Corn.....*Zea mays*.

Sorghum.....*Holcus sorghum*.

Pearl millet.....*Pennisetum glaucum*.

## Ornamentals:

## Perennial—

Eulalia.....*Miscanthus sinensis*.

## Wild grasses:

## Perennial—

Wild sugar cane.....*Saccharum narenga* (S. P. I. No. 38332).<sup>a</sup>

## Annuals—

Bull-grass.....*Paspalum boscianum*.

Crab-grass.....*Syntherisma sanguinalis*.

Yellow foxtail.....*Chaetochloa lutescens*.

Giant foxtail.....*Chaetochloa magna*.

Barnyard grass.....*Echinochloa crusgalli*.

Panicum.....*Panicum dichotomiflorum*.

Brachiaria.....*Brachiaria platyphylla*.

<sup>a</sup> A variety of *Saccharum narenga* is cultivated in China, according to Mr. Frank N. Meyer

The grasses tested with negative results are given in the following list:

Cultivated crops—

Perennials—

Madake bamboo.....	<i>Phyllostachys quilioides</i> .
Edible bamboo.....	<i>Phyllostachys pubescens</i> .
Para grass.....	<i>Panicum barbinode</i> .
Napier grass.....	<i>Pennisetum purpureum</i> var.
Merker grass.....	Do.
Australian giant-grass.....	Do.
Johnson grass.....	<i>Holcus halepensis</i> .

Annuals—

Wheat, Power Fife (C. I. 3697).....	<i>Triticum aestivum</i> .
Oats, Swedish Select (C. I. 134).....	<i>Avena sativa</i> .
Rye, Von Runker No. 2 (C. I. 174).....	<i>Secale cereale</i> .
Barley, Marionet.....	<i>Hordeum vulgare</i> .
Rice, Blue Rose (C. I. 1962).....	<i>Oryza sativa</i> .
Teosinte.....	<i>Euchlaena mexicana</i> .
Redtop.....	<i>Agrostis palustris</i> .
Timothy.....	<i>Phleum pratense</i> .
Bluegrass.....	<i>Poa pratensis</i> .
Ragi millet.....	<i>Eleusine coracana</i> .

Ornamentals—

Perennials—

Variegated Eulalia.....	<i>Miscanthus sinensis variegatus</i> .
Do.....	<i>Miscanthus sinensis zebrinus</i> .

Annual—

Job's-tears.....	<i>Coix lachryma-jobi</i> .
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Wild grasses:

Perennials—

Broom sedge.....	<i>Andropogon virginicus</i> .
Broom sedge.....	<i>Andropogon ellipticus</i> .
Little bluestem.....	<i>Andropogon scoparius</i> .
Indian reed.....	<i>Sorghastrum nutans</i> .
Gama grass.....	<i>Tripsacum dactyloides</i> .

Annuals—

Green foxtail.....	<i>Chaetochloa viridis</i> .
Red sprangletop.....	<i>Leptochloa filiformis</i> .

It is noticeable that although plants have been selected for experiment from the entire grass family, the ones which proved susceptible are without exception confined to the tribes Paniceae, Andropogoneae, and Tripsaceae. In his phylogenetic arrangement of the genera of grasses, Hitchcock (9) divides the grass family into 13 tribes and places the 3 tribes here mentioned together as representing the highest type of development. It is significant that this arrangement, based on morphological characters, should prove so regular with respect to susceptibility to this disease. Since it is to be expected that closely related species are more likely to be affected by the same diseases than species located remotely from one another, the relationships are corroborated in an interesting and novel manner.

The possibility of the existence of more than one type of mosaic among the grasses has been mentioned. A type of mottling in the edible bamboo (*Phyllostachys pubescens*) from China resembles our mosaic very strikingly. It was first observed in one plant out of a lot of four being held in the detention house at Washington. Subsequently two other plants became affected. The writers have no experimental evidence that this is an infectious disease. It was found impossible to infect this species of bamboo with the sugar-cane mosaic. Furthermore, it is not nearly related to any other grass found susceptible to our mosaic but stands at the extreme opposite end of the list of tribes of the grass family.

A specific mosaic of *Nicotiana viscosum* distinct from the mosaic of *N. tabacum* has been reported by Allard (1), who states that the mosaic of

*N. viscosum* is more difficult to transmit artificially than the common tobacco mosaic. *Datura stramonium* was the only solanaceous plant found susceptible to both mosaic diseases. Allard suggests that results of inoculations with tobacco mosaic by various European investigators which are inconsistent with his own may be due to the existence of different types of mosaic in the Solanaceae.

### COMPOSITE RECORD AND RESULTS OF EXPERIMENTS

The technic of the work here reported has been exactly the same as that described in a previous paper (3). All inoculations were made with one-half to 2 cc. of cell sap, obtained by squeezing young stalks in a powerful press under mineral oil. The virus or healthy sap was injected near the growing point by means of Leur all-glass hypodermic syringes provided with Yale 24-gauge needles. The results of the experiments are given in support of the facts presented in the preceding pages. In order to save space, composite records of the experiments are shown in tabular form.

Table I includes tests made for artificial transmission.

TABLE I.—Composite record of tests of the artificial transmission of grass mosaic

Species.	Number of plants	Date inoculated	Inoculum.	Results.	
				Date examined.	Condition.
<i>Saccharum officinarum</i> var. Louisiana Purple	4	Oct. 4, 1920	Virus from sugar cane variety B6450.	Oct. 18, 1920	3 mosaic.
Do .....	4	.....do.....	Sap from healthy sugar variety B6450.	Nov. 15, 1920	All healthy.
Do .....	4	.....do.....	Virus from B6450 passed through Berkeley filter.	Oct. 18, 1920	3 mosaic.
Do .....	4	.....do.....	Virus from B6450 mixed with equal part of 0.2 per cent phenol	.....do.....	1 mosaic.
Do .....	4	.....do.....	Virus from B6450 mixed with equal part of 0.05 per cent CuSO <sub>4</sub> .	Nov. 15, 1920	All healthy.
Do .....	4	.....do.....	Virus from B6450 mixed with equal part of 5 per cent formalin.	.....do.....	Do.
Do .....	4	.....do.....	Virus from B6450 mixed with 1 per cent Carrol Dakin solution	.....do.....	Do.
Do .....	4	.....do.....	Virus from B6450 mixed with 0.1 per cent HgCl <sub>2</sub> .	.....do.....	Do.
Do .....	4	.....do.....	Virus from B6450 diluted 1/100 with distilled water	.....do.....	Do.
Do .....	4	.....do.....	Virus from B6450 diluted 1/1,000 with distilled water.	.....do.....	Do.
Do .....	4	.....do.....	Virus from B6450 diluted 1/10,000 with distilled water.	.....do.....	Do.
Do .....	8	Aug. 4, 1920	Virus from Louisiana Purple	Aug. 28, 1920	8 mosaic.
<i>Zea mays</i> var. U. S. Selection No. 18a.	3	.....do.....	.....do.....	Sept. 29, 1920	All healthy.
<i>Holcus sorghum</i> .....	2	.....do.....	.....do.....	Aug. 28, 1920	2 mosaic.
<i>Syntherisma sanguinalis</i> .....	6	.....do.....	.....do.....	.....do.....	5 mosaic.
<i>Coxs lachryma-jobi</i> .....	2	.....do.....	.....do.....	Sept. 29, 1920	Both healthy.
<i>Pennisetum glaucum</i> .....	6	.....do.....	.....do.....	Aug. 28, 1920	6 mosaic.
<i>Saccharum officinarum</i> var. Louisiana Purple.	8	.....do.....	Sap from healthy sugar cane, variety Louisiana Purple.	Sept. 29, 1920	All healthy.
<i>Zea mays</i> .....	3	.....do.....	.....do.....	.....do.....	Do.
<i>Holcus sorghum</i> .....	2	.....do.....	.....do.....	.....do.....	Do.
<i>Syntherisma sanguinalis</i> .....	6	.....do.....	.....do.....	.....do.....	Do.
<i>Coxs lachryma-jobi</i> .....	2	.....do.....	.....do.....	.....do.....	Do.
<i>Pennisetum glaucum</i> .....	6	.....do.....	.....do.....	.....do.....	Do.

It will be noticed that in the experiments the virus was treated in various ways. In at least one experiment virus which had been passed through a Berkefeld filter<sup>4</sup> caused infection in 75 per cent of the plants inoculated. Other similar experiments have failed to give such convincing results, but owing to the fact that control plants inoculated with untreated virus also show a low percentage of infection, indicating a less potent virus, the writers are inclined to attach considerable importance to the successful experiment and believe that the virus will pass through certain diatomaceous earth filters.

In some experiments the virus was shaken in bottles with various bacteriacidal chemicals one hour before being injected into the plants. With one exception none of the virus treated in this way caused infection. One plant in a series of four inoculated with virus treated with weak phenol solution became mosaic in 14 days. The same lot of virus diluted with distilled water in various proportions gave negative results. Virus capable of causing infection when used immediately after being expressed from diseased stalks was found in one experiment to be without effect when injected 24 hours later. The virus of grass mosaic is less stable or more sensitive to the influence of its environment than that of many other similar diseases, notably the tobacco mosaic. In these experiments it has been found very refractory and difficult of physical manipulation or chemical treatment without loss of virulence.

The results of the experiments on insect transmission of the disease are given in Table II. "Virulent" insects were obtained from mosaic sugar cane or from mosaic sorghum artificially inoculated with virus from sugar cane. "Nonvirulent" insects were from healthy cane or sorghum. These experiments were performed in insect-proof compartments in a mosaic-infested greenhouse.

TABLE II.—Composite record of the tests of the insect transmission of the grass mosaic

Species.	Number of plants.	Date exposed	Character.	Insect.	Results.	
					Date examined.	Condition
<i>Saccharum officinarum</i> var. Louisiana Purple.	3	Feb. 4, 1920	Virulent...	<i>Aphis maidis</i> ..	Feb. 28, 1920	2 mosaic.
Do.....	3	.....do....	Nonvirulent	.....do....	Mar. 14, 1920	All healthy.
Do.....	6	.....do....	Virulent.	<i>Draeculacephala molispes</i>	.....do.....	Do.
Do.....	6	.....do....	Nonvirulent	do....	do....	Do.
<i>Zea mays</i> var. U. S. Selection No 182	6	Mar. 12, 1920	Virulent.	<i>Aphis maidis</i>	Apr. 6, 1920	4 mosaic.
Do.....	6	.....do....	Nonvirulent	do.....	May 1, 1920	All healthy.
Do.....	20	Mar. 30, 1920	Virulent..	do....	May 28, 1920	15 mosaic.
Do.....	20	.....do....	Nonvirulent	do....	.....do.....	All healthy.
<i>Triticum sativum</i> var. Power File.	50	May 20, 1920	Virulent....	do....	Aug. 1, 1920	Do.
Do.....	50	.....do....	Nonvirulent	do.....	.....do....	Do.
<i>Hordeum vulgare</i> var. Marionet.	50	.....do....	Virulent.	do....	.....do....	Do.
Do.....	50	.....do....	Nonvirulent	do.....	.....do....	Do.
<i>Secale cereale</i> var. Von Runker No 2.	50	.....do....	Virulent...	do.....	.....do....	Do.
Do.....	50	.....do....	Nonvirulent	do.....	.....do....	Do.
<i>Avena sativa</i> var. Swedish Select.	50	.....do....	Virulent....	do.....	.....do....	Do.
Do.....	50	.....do....	Nonvirulent	do.....	.....do....	Do.

<sup>4</sup> This was a rather coarse filter. When immersed in water it withstood a pressure of only 3 pounds before passing bubbles.

TABLE II.—Composite record of the tests of the insect transmission of the grass mosaic—Continued

Species.	Number of plants.	Date exposed.	Character.	Insect.	Results.	
					Date examined.	Condition.
<i>Oryza sativa</i> var. C I No. 1962.	50	May 20, 1920	Virulent...	<i>Aphis maidis</i> .	Aug. 1, 1920	All healthy.
Do.	50	do.	Nonvirulent	do.	do.	Do.
<i>Eleusine coracana</i> var. S. P. I. No. 43190.	50	do.	Virulent	do.	do.	Do.
Do.	50	do.	Nonvirulent	do.	do.	Do.
<i>Agrostis palustris</i> ...	40	May 15, 1920	Virulent	do.	do.	Do.
Do.	40	do.	Nonvirulent	do.	do.	Do.
<i>Poa pratensis</i> ...	40	do.	Virulent	do.	do.	Do.
Do.	40	do.	Nonvirulent	do.	do.	Do.
<i>Holcus halepenses</i> ...	40	do.	Virulent	do.	do.	Do.
Do.	40	do.	Nonvirulent	do.	do.	Do.
<i>Phleum pratense</i> ...	40	do.	Virulent	do.	do.	Do.
Do.	40	do.	Nonvirulent	do.	do.	Do.
<i>Coix lachryma-jobi</i>	6	do.	Virulent	do.	June 15, 1920	All mottled; not clear case of mosaic.
Do.	6	do.	Nonvirulent	do.	do.	All healthy.
<i>Miscanthus sinensis</i>	16	May 20, 1920	Virulent	do.	July 15, 1920	5 mosaic.
Do.	6	do.	Nonvirulent	do.	do.	All healthy.
<i>Pennisetum purpureum</i> .	4	May 30, 1920	Virulent	do.	June 30, 1920	Do.
Do.	4	do.	Nonvirulent	do.	do.	Do.
Merker grass	4	do.	Virulent	do.	do.	Do.
Do.	4	do.	Nonvirulent	do.	do.	Do.
Australian giant grass.	4	do.	Virulent	do.	July 6, 1920	Do.
Do.	4	do.	Nonvirulent	do.	do.	Do.
<i>Syntherisma sanguinalis</i> .	10	June 1, 1920	Virulent	do.	July 29, 1920	6 mosaic.
Do.	10	do.	Nonvirulent	do.	do.	All healthy.
<i>Zea mays</i> var. U. S. Selection No 182	50	do.	Virulent	do.	July 28, 1920	2 mosaic.
Do.	50	do.	Nonvirulent	do.	do.	All healthy.
<i>Paspalum boscianum</i> .	3	July 31, 1920	Virulent	do.	Aug 31, 1920	3 mosaic.
Do.	3	do.	Nonvirulent	do.	do.	All healthy.
<i>Saccharum officinarum</i> var. Louisiana Purple	10	Aug 3, 1920	Virulent	do.	Aug 28, 1920	3 mosaic.
Do.	10	do.	Nonvirulent	do.	Sept. 15, 1920	All healthy.
<i>Zea mays</i> ...	10	do.	Virulent	do.	Aug. 28, 1920	3 mosaic.
Do.	10	do.	Nonvirulent	do.	Sept. 15, 1920	All healthy.
<i>Holcus sorghum</i> ...	5	do.	Virulent	do.	Aug. 28, 1920	5 mosaic.
Do.	5	do.	Nonvirulent	do.	Sept. 15, 1920	All healthy.
<i>Coix lachryma-jobi</i> ...	5	do.	Virulent	do.	do.	Do.
Do.	5	do.	Nonvirulent	do.	do.	Do.
<i>Syntherisma sanguinalis</i> .	5	do.	Virulent	do.	Aug. 28, 1920	5 mosaic.
Do.	5	do.	Nonvirulent	do.	Sept. 15, 1920	All healthy.
<i>Miscanthus sinensis</i> .	2	do.	Virulent	do.	Oct. 3, 1920	2 mosaic.
Do.	2	do.	Nonvirulent	do.	do.	All healthy.
<i>Saccharum officinarum</i> var. Louisiana Purple.	10	do.	Virulent	<i>Kolla similis</i>	Sept. 15, 1920	Do.
Do.	10	do.	Nonvirulent	do.	Sept. 9, 1920	Do.
<i>Zea mays</i> ...	10	do.	Virulent	do.	do.	Do.
Do.	10	do.	Nonvirulent	do.	do.	Do.
<i>Holcus sorghum</i> ...	5	do.	Virulent	do.	do.	Do.
Do.	5	do.	Nonvirulent	do.	do.	Do.
<i>Coix lachryma-jobi</i> ...	5	do.	Virulent	do.	do.	Do.
Do.	5	do.	Nonvirulent	do.	do.	Do.
<i>Syntherisma sanguinalis</i> .	5	do.	Virulent	do.	do.	Do.
Do.	5	do.	Nonvirulent	do.	do.	Do.
<i>Miscanthus sinensis</i> .	2	do.	Virulent	do.	do.	Do.
Do.	2	do.	Nonvirulent	do.	do.	Do.
<i>Saccharum officinarum</i> var. Louisiana Purple.	10	do.	Virulent	<i>Draculacephala molipes</i> .	do.	Do.
Do.	10	do.	Nonvirulent	do.	do.	Do.
<i>Zea mays</i> ...	10	do.	Virulent	do.	do.	Do.
Do.	10	do.	Nonvirulent	do.	do.	Do.
<i>Holcus sorghum</i> ...	5	do.	Virulent	do.	Sept. 15, 1920	Do.
Do.	5	do.	Nonvirulent	do.	do.	Do.
<i>Coix lachryma-jobi</i> ...	5	do.	Virulent	do.	do.	Do.
Do.	5	do.	Nonvirulent	do.	do.	Do.

TABLE II.—Composite record of the tests of the insect transmission of the grass mosaic—Continued

Species.	Number of plants.	Date exposed.	Character.	Insect.	Results.	
					Date examined.	Condition.
<i>Syntherisma sanguinalis</i>	5	Aug. 3, 1920	Virulent....	<i>Draeculacephala molipes</i>	Sept. 15, 1920	All healthy.
Do	5	do	Nonvirulent	do	do	Do.
<i>Miscanthus sinensis</i>	2	do	Nonvirulent	do	do	Do.
Do	2	do	Nonvirulent	do	do	Do.
<i>Brachiaria platyphylla</i>	7	July 13, 1920	Virulent	<i>Aphis maidis</i>	Aug. 13, 1920	5 mosaic.
Do	5	do	Nonvirulent	do	do	All healthy.
<i>Syntherisma sanguinalis</i>	2	Aug. 16, 1920	Virulent	do	Sept. 20, 1920	Both mosaic.
Do	2	do	Nonvirulent	do	do	Both healthy.
<i>Paspalum boscianum</i>	2	do	Virulent	do	do	Both mosaic.
Do	2	do	Nonvirulent	do	do	Both healthy
<i>Leptochloa filiformis</i>	1	Aug. 17, 1920	Virulent	do	do	Healthy.
Do	1	do	Nonvirulent	do	do	Do.
<i>Saccharum officinarum</i>	3	Sept. 2, 1920	Virulent	<i>Draeculacephala molipes</i>	Nov. 1, 1920	All healthy.
<i>Zea mays</i>	3	do	do	do	do	Do.
<i>Holcus sorghum</i>	2	do	do	do	do	Do.
<i>Coxiachryma-jobi</i>	2	do	do	do	do	Do.
<i>Syntherisma sanguinalis</i>	2	do	do	do	do	Do.
<i>Miscanthus sinensis</i>	2	do	do	do	do	Do.
<i>Leptochloa filiformis</i>	6	do	do	<i>Aphis maidis</i>	Nov. 18, 1920	Do.
<i>Saccharum officinarum</i>	10	Oct. 1, 1920	Exposed on ledge <sup>a</sup>	do	Feb. 25, 1921	9 mosaic.
<i>Holcus sorghum</i>	15	Feb. 3, 1921	do	do	Apr. 2, 1921	6 mosaic.
<i>Chaetochloa lutescens</i>	3	do	do	do	do	3 mosaic.
<i>Syntherisma sanguinalis</i>	4	do	do	do	do	All healthy.
<i>Paspalum boscianum</i>	6	do	do	do	do	6 mosaic.
<i>Saccharum officinarum</i>	3	do	Virulent	<i>Draeculacephala molipes</i>	May 6, 1921	All healthy.
Do	3	do	Nonvirulent	do	do	Do.
<i>Echinochloa crus-galli</i>	3	Apr. 7, 1921	Exposed on ledge <sup>a</sup>	do	May 25, 1921	2 mosaic.
<i>Miscanthus sinensis</i>	3	Apr. 13, 1921	do	do	June 15, 1921	Healthy.
<i>Miscanthus sinensis</i>	4	do	do	do	do	Do.
<i>Sorghastrum nutans</i>	3	do	do	do	do	All healthy.
<i>Chaetochloa magna</i>	4	do	do	do	do	2 mosaic.
<i>Paspalum boscianum</i>	4	May 25, 1921	Virulent	<i>Aphis maidis</i>	June 20, 1921	4 mosaic.
<i>Chaetochloa magna</i>	2	do	do	do	do	2 mosaic.
<i>Chaetochloa lutescens</i>	8	do	do	do	do	All healthy.
<i>Andropogon ellipticus</i>	6	do	do	do	do	Do.
<i>Andropogon virginicus</i>	6	do	do	do	do	Do.
<i>Andropogon scoparius</i>	6	do	do	do	do	Do.
<i>Syntherisma sanguinalis</i>	3	do	do	do	do	2 mosaic.
<i>Brachiaria platyphylla</i>	4	do	do	do	do	All healthy.
<i>Panicum dichotomiflorum</i>	2	do	do	do	do	2 mosaic.
<i>Sorghastrum nutans</i>	2	do	do	do	do	Both healthy.

<sup>a</sup> These plants were merely exposed to natural infection by being placed on a ledge in a greenhouse containing infected plants with which they were not in direct contact.

The percentage of plants infected in both artificial-inoculation and insect-transmission experiments is not high in many cases, but the results are conclusive owing to the conditions under which all experiments were performed. All grasses for experimental purposes were grown in a greenhouse at Washington hundreds of miles from any known

cases of natural infection. When suitable for inoculation they were removed to another greenhouse where the inoculations were performed. In practically every case equal numbers of plants were inoculated with nonvirulent juice as controls. Insect-transmission experiments were conducted in cages in a third greenhouse with plant material raised in the greenhouse first mentioned. In these experiments also equal numbers of control plants were placed in adjoining cages. No case of mosaic has occurred among the control plants in any experiment performed.

In some experiments a method of handling aphids was developed by which the tedious operation of lifting individual insects with a camel's hair brush was eliminated. Small bits of infected sorghum leaves covered with the insects were clipped off with scissors and placed on or tied to the plants to be tested. Similar pieces of leaves from which the insects had been carefully removed were tied to a series of control plants, and healthy leaves with nonvirulent aphids were tied to a second series of control plants. Although it is necessary to run two sets of control plants in order to check all factors properly, this method was found to be of great convenience. The experiments noted in Table III were performed in the manner described.

TABLE III.—Results of a test of the insect transmission of grass mosaic by the new method

Species	Number of plants.	Date exposed.	Treatment	Results.	
				Date examined.	Condition
<i>Saccharum officinarum</i>	3	Aug. 3, 1920	Aphids on mosaic leaf	Aug. 24, 1920	2 mosaic.
var. Louisiana Purple	3	do ..	Mosaic leaf, no insects	Sept. 15, 1920	All healthy.
Do .. .	3	do ..	Aphids on healthy leaf	do ..	Do
<i>Zea mays</i>	3	do ..	Aphids on mosaic leaf	Aug. 30, 1920	2 mosaic
Do .. .	3	do ..	Mosaic leaf, no insects	Sept. 15, 1920	All healthy.
Do .. .	3	do ..	Aphids on healthy leaf	do ..	Do
<i>Holcus sorghum</i>	2	do ..	Aphids on mosaic leaf	Aug. 26, 1920	Both mosaic
Do .. .	2	do ..	Mosaic leaf, no insects	Sept. 15, 1920	Both healthy
Do .. .	2	do ..	Aphids on healthy leaf	do ..	Do
<i>Miscanthus sinensis</i>	2	do ..	Aphids on mosaic leaf	Aug. 30, 1920	Mosaic.
Do .. .	1	do ..	Mosaic leaf, no insects	Sept. 15, 1920	Healthy
Do .. .	1	do ..	Aphids on healthy leaf	do ..	Do
<i>Syntherisma sanguinalis</i>	2	do ..	Aphids on mosaic leaf	Aug. 30, 1920	Both mosaic.
Do .. .	2	do ..	Mosaic leaf, no insects	Sept. 15, 1920	Both healthy.
Do .. .	2	do ..	Aphids on healthy leaf	do ..	Do

Although a considerable number of species of insects have been tested for their ability to act as carriers of grass mosaic, it has been demonstrated only for *Aphis maidis* (*A. adusta*) by the writers. Our results on the transmission of the disease with this insect have been corroborated by Ledebuer (13),<sup>5</sup> who has also succeeded in transmitting it by the use of *A. sacchari*. Owing to its abundance and omnivorous habits, *A. maidis* has been found very convenient for use in ascertaining the susceptibility to mosaic of various grasses. Several grasses, notably corn and millet, have been found by the writers to be very difficult or even impossible to infect by artificial inoculation, but it is comparatively easy to bring about infection by the use of virulent corn aphids. The corn aphids, of course, do not feed on all the grasses equally well, and on some it was found quite impossible to establish them. They are not abundant on sugar cane.

<sup>5</sup> Since this paper was prepared, nearly two years ago, the work reported in Ledebuer's preliminary report has appeared. WILBRINK, G. "EEN ONDERZOEK NAAR DE VERBREIDING DER GELESTREFTHEID DOOR BLADLUZEN." In *Archief Suikerindus. Nederl.-Indie*, Meded. v. h. proefst. v. d. Javasuikerindus. 1922, no. 20, p. 413-456. Dr. Wilbrink confirms *A. maidis* as a vector of mosaic but not *A. sacchari*.

*A. maidis* has been reported on sugar cane, however, (5, 8, 18)<sup>6</sup>, and on other crops from many sugar-cane regions.

While it is usually difficult to establish *Aphis maidis* on sugar cane in the greenhouse by transplanting them from other grasses, they sometimes migrate naturally to the cane and are found on it in large numbers. One of the worst infestations of any grass by *A. maidis* ever seen by the writers was on sugar-cane seedlings about 5 months old. It was brought to our attention by Dr. B. T. Galloway, who was growing the seedlings in one of the Washington greenhouses. In some of our experiments where *A. maidis* failed to become established and disappeared within 2 or 3 days, the plants nevertheless were infected by them and showed symptoms after the usual incubation period of 14 to 20 days. Corn, sorghum, and pearl millet are favorite food plants for *A. maidis*, which is frequently found on them in enormous numbers. The insect is an ideal carrier in the case of these three crops. Several species of leafhoppers have been held under suspicion as vectors of grass mosaic on account of strong indirect evidence, but no positive proof of such capacity on their part was developed.

#### SOME ECONOMIC ASPECTS OF GRASS MOSAIC

##### TESTS WITH CANE VARIETIES OF THE NORTH INDIA TYPE

Practically all of the well-known varieties of sugar cane are susceptible to grass mosaic. Prof. F. S. Earle noticed, however, that the so-called Kavangire, a variety of the slender North India type, was not affected under conditions favorable to the transmission of the disease (15). Later observations have indicated that the Uba, grown extensively in Natal, and Cayana No. 10, in the sirup sections of this country, are apparently immune. These varieties are of the same type, and Prof. Earle has declared that Kavangire is identical with the old well-known Uba (6). A collection of varieties of this type from various parts of the world was brought together in Washington to determine whether immunity to mosaic is characteristic of the whole group. They were placed in a greenhouse exposed to natural infection, with the results indicated in Table IV.

TABLE IV.—Susceptibility to the mosaic disease of the varieties of the North India type of sugar cane

Variety.	Date exposed.	Result.	
		Date examined	Condition.
Uba .....	Jan. 6, 1921	July 1, 1921	All healthy.
Kavangire .....	do	do	Do.
Cayana No. 10 .....	do	do	Do.
<i>Saccharum narenga</i> (S.P.I. No. 38332) .....	do	Jan. 25, 1921	All mosaic.
Khera (S.P.I. No. 33242) .....	do	do	Do.
Merthi (S.P.I. No. 33243) .....	do	July 1, 1921	All healthy.
Kinar (S.P.I. No. 33245) .....	do	do	Do.
Chikusho (S.P.I. No. 29106) .....	do	Jan. 25, 1921	All mosaic.
Var. from Kagawa Ken (S.P.I. No. 29107) .....	do	do	Do.
Kikaigashima (S.P.I. No. 29108) .....	do	do	Do.
Oshima (S.P.I. No. 29109) .....	do	July 1, 1921	All healthy.
Chikucha (S.P.I. No. 30404) .....	do	Jan. 25, 1921	All mosaic.

<sup>6</sup> Mr. A. C. Baker reports *Aphis maidis* on sugar cane in the quarantine greenhouse of the Federal Horticultural Board at Washington, March 11, 1920 (in letter to the writers, December 2, 1921), and Mr. Geo. W. Wolcott reports finding this species on sugar cane in Porto Rico (in letter to Dr. C. O. Townsend, December 30, 1921).



With the exception of the plants labeled *Saccharum narenga*, these varieties are all very similar, and some of them may be identical. They are of the slender North India type and probably have a common origin, but this experiment proves that not all varieties of this type are immune to mosaic. Varieties from India, China, and Japan proved to be susceptible. They are apparently scarcely injured by the disease. The leaf symptoms are much less conspicuous than in the thick-stalked varieties of sugar cane, and there is no evidence of stunting. In this respect they are like the well-known Java seedling varieties resulting from crosses between the Chunnee ♂ and Striped Preanger ♀ and between Chunnee ♂ and Black Cheribon ♀.

#### TESTS WITH CORN VARIETIES

That some varieties of corn are severely injured by grass mosaic was shown by the senior writer in 1920 (4). In 1921 a large number of corn varieties were tested in southern Georgia for immunity or resistance to the disease. The first experiment included 40 varieties of field, sweet, and pop corn from all of the corn sections of the United States.<sup>7</sup> About 25 plants of each variety were grown near the center of a field of first ratoon Louisiana Purple sugar-cane plants, more than half of which were mosaic. Seeds were planted in the field on April 15, 1921. On July 15, 1921, the plot was examined and notes were taken on the percentage of infected plants in each variety. The results are given in Table V.

TABLE V.—Results of tests for resistance to mosaic of varieties of corn planted on April 15, 1921

Variety.	Source of seed.	Per- cent- age of mosaic on July 15, 1921.	Variety.	Source of seed	Per- cent- age of mosaic on July 15, 1921.
Native .....	Georgia .....	20	Clarage (U. S. Selection No. 125) .....	Maryland ...	0
Pope Prolific .....	Florida .....	20	Boone County (U. S. Selection No. 159) .....	Nebraska ...	0
U. S. Selection No. 165 .....	Texas .....	35	St. Charles (U. S. Selec- tion No. 202) .....	.. do.....	0
U. S. Selection No. 170 .....	.. do .....	40	Lancaster Surecrop .....	Illinois .....	0
Laguna .....	.. do .....	10	U. S. Selection No. 160 .....	California .....	15
Brazos .....	do .....	5	Orange County Prolific .....	.. do .....	15
Arlington Prolific .....	Mississippi .....	17	U. S. Selection No. 204 .....	South Dakota .....	10
Red Cob .....	do .....	10	Northwestern .....	North Dakota .....	0
Millpond Prolific .....	Georgia .....	20	Gehu .....	.. do .....	0
Whately Prolific .....	do .....	15	Pearl .....	.. do .....	0
Gerrick .....	South Carolina .....	12	U. S. Selection No. 133 .....	Wisconsin .....	0
U. S. Selection No. 201 .....	Arkansas .....	12	Hall Gold Nugget (U. S. Selection No. 193) .....	New York .....	0
Cuban Yellow .....	Florida .....	20	Arlington Peruvians .....	Virginia .....	15
Station Yellow .....	Alabama .....	0	Pueblo Black .....	New Mexico .....	0
Singleton .....	Texas .....	0	White Rice .....	Virginia .....	4
Huffman .....	Tennessee .....	0	Yellow Pearl .....	.. do .....	4
U. S. Selection No. 230 .....	Virginia .....	10	Hull-less .....	Michigan .....	0
Boone County (U. S. Selection No. 119) .....	.. do .....	10	Golden Bantam .....	Virginia .....	0
U. S. Selection No. 120 .....	.. do .....	0	Country Gentleman .....	.. do .....	0
U. S. Selection No. 182 .....	do .....	10			
Woodburn (U. S. Selec- tion No. 77) .....	Ohio .....	0			

Twenty-three of the 40 varieties became affected with mosaic in this experiment. All varieties from the Southern States excepting 4, which later proved susceptible, were more or less affected by the disease; but

<sup>7</sup> The seed of these varieties was furnished through the courtesy of the Office of Cereal Investigations, U. S. Department of Agriculture.

the varieties from Northern and Western States were conspicuously free from it. This result may be due in part to the fact that the northern and western varieties were subnormal in vigor. They were for the most part only half as tall as the southern varieties. It is now well known that a slight shortening of the accustomed length of day may check vegetative growth and hasten maturity in some varieties of plants (7), and this fact may account for the subnormal development of these varieties. It has been our experience that stunted plants are more difficult to infect experimentally than normal ones. The explanation for the low percentage of infection in the varieties which were out of their proper environment may therefore be due to this fact rather than to any innate character of resistance.

A second experiment on a much larger scale with southern varieties of field corn was started on May 15, one month after the first experiment. About 3 acres of corn, approximately equally divided among 17 varieties, was planted in a field immediately adjacent to badly diseased first ratoons of Louisiana Purple sugar cane. On July 15 the whole planting was carefully examined and a large proportion of all varieties was discovered to be already affected by the disease. The percentage of diseased plants on that date is given in Table VI.

TABLE VI.—Results of tests for resistance to mosaic of varieties of southern field corn planted on May 15, 1921

Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.	Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.
Native .....	Georgia ..	15	Whatley Prolific....	Georgia ..	40
Pope Prolific .....	Florida ..	75	Gerrick .....	South Carolina ..	50
U. S Selection No. 165 ..	Texas .....	30	U. S Selection No. 201 ..	Arkansas... ..	60
U. S Selection No. 170 ..	.. do .....	50	Cuban Yellow .....	Florida .....	45
Laguna .....	.. do .....	24	Station Yellow .....	Alabama .....	66
Brazos .....	.. do .....	40	Singleton .....	Texas .....	60
Arlington Prolific.....	Mississippi ..	50	U. S Selection No. 230 ..	Virginia.....	45
Red Cob .....	.. do .....	25	Boone County (U. S	....do.....	70
Milpond Prolific.....	Georgia .....	85	Selection No. 119)		

A much higher percentage of infection was found in these plants than in the same varieties planted one month earlier. All of the corn was heavily infested with *Aphis maidis*, but no other insect was noticeably abundant.

In order to obtain data on the damage inflicted by this disease, 10 each of mosaic and apparently healthy plants in each variety were tagged for identification at harvest time. It is realized that many plants marked "healthy" on July 15 may have become infected before harvest, so that any decrease in yield indicated by this method does not represent the real extent of the loss sustained. The loss due to mosaic is without question greater than that indicated by our data. All tagged plants with legible inscriptions were harvested on September 22, and the ears were examined and weighed. The number of ears, total weight, and average weight of ears of healthy and mosaic plants for each variety are given in Table VII.

TABLE VII.—Weight of healthy and mosaic ears of corn varieties planted May 15, 1921

Variety.	Source of seed.	Condition.	Ears on 10 plants.	Weight.		Decrease due to mosaic.
				Total.	Average.	
			Number	Gm.	Gm.	Per cent.
Native.....	Georgia.....	Healthy..	5	535	107	.....
Do.....	do.....	Mosaic..	7	470	67.1	37.3
Pope Prolific.....	Florida.....	Healthy..	8	822	102.7	.....
Do.....	do.....	Mosaic..	5	402	80.4	21.8
U. S. Selection No. 165	Texas.....	Healthy..	4	665	166.2	.....
Do.....	do.....	Mosaic..	9	780	82.2	50.6
U. S. Selection No. 170..	do.....	Healthy..	6	995	165.8	.....
Do.....	do.....	Mosaic..	6	830	138.3	16.6
Laguna.....	do.....	Healthy..	5	765	153.0	.....
Do.....	do.....	Mosaic..	5	735	147.0	4.0
Brazos.....	do.....	Healthy..	6	1,115	186.0	.....
Do.....	do.....	Mosaic..	7	815	116.4	37.5
Arlington Prolific...	Mississippi.	Healthy..	10	1,100	110.0	.....
Do.....	do.....	Mosaic..	6	470	78.2	29.0
Red Cob.....	do.....	Healthy..	4	555	138.7	.....
Do.....	do.....	Mosaic..	5	640	128.0	7.8
Millpond Prolific...	Georgia.....	Healthy..	7	750	107.1	.....
Do.....	do.....	Mosaic..	10	595	59.5	44.5
Whately Prolific.....	do.....	Healthy..	7	790	112.8	.....
Do.....	do.....	Mosaic..	11	880	80.0	29.1
Gerrick.....	South Carolina	Healthy..	7	580	82.9	.....
Do.....	do.....	Mosaic..	12	890	74.1	20.7
U. S. Selection No. 201	Arkansas.....	Healthy..	8	910	113.5	.....
Do.....	do.....	Mosaic..	6	518	86.3	24.0
Cuban Yellow.....	Florida.....	Healthy..	5	815	163.0	.....
Do.....	do.....	Mosaic..	12	1,140	95.0	41.8
Station Yellow.....	Alabama.....	Healthy..	8	900	112.2	.....
Do.....	do.....	Mosaic..	8	755	94.3	16.0
Singleton.....	Texas.....	Healthy..	7	784	112.0	.....
Do.....	do.....	Mosaic..	6	670	111.6	.4
U. S. Selection No. 230	Virginia.....	Healthy..	2	318	159.0	.....
Do.....	do.....	Mosaic..	3	317	102.3	35.7
Boone County (U. S. Selection No. 119)...	do.....	Healthy..	2	205	102.5	.....
Do.....	do.....	Mosaic..	6	435	72.5	29.3

This data indicates that with corn, as with sugar cane, some susceptible varieties tolerate the disease without greatly injurious results, while other varieties are severely injured by it. Plate 1, B, illustrates a variety not noticeably injured by mosaic. It was pointed out in a former publication (4) that in the case of "White Creole" corn in Louisiana the ears of affected plants are not only stunted but they are poorly filled, some being quite sterile. The rows of kernels in affected ears are likely to be very irregular as compared with the straight parallel rows of normal ears. In nearly all varieties in the present experiments the same condition was apparent and is well illustrated in Plate 1, A, and also in Plates 2 to 4. It will be noticed that some of the ears marked "healthy" are small and some show irregularity in the rows, but by no means to the same degree as in the mosaic ears. This may be due partly to the fact previously noted that plants labeled healthy in the field may have become infected later. The writers believe that grass mosaic is a serious disease of corn where conditions are favorable for infection. Since the disease is apparently not transmitted in the corn seed, as will be briefly considered

later, the infection must come each year from some diseased perennial grass. In all sugar-cane plantations, corn is invariably used in the crop rotation, and one of the required conditions for infection of corn is present if the disease exists in the nearby cane.

Corn mosaic has now been reported from Porto Rico, the United States, Guam, the Hawaiian Islands, and Trinidad.

In a publication by Kunkel (12), just received from Hawaii, a very complete cytological investigation of corn mosaic is recorded. Certain large bodies found in the cells of diseased tissues are described and suggested as the possible cause of the disease. Such cell inclusions have been noticed and recorded by early investigators of mosaic, but this was not mentioned and possibly was overlooked by Kunkel. Practically identical bodies were carefully described and accurately illustrated by Iwanowski (10) in his researches on tobacco mosaic published nearly 20 years ago. The latter, however, was cautious about ascribing to these bodies any etiological significance merely on the basis of their association with the disease. The paper by Kunkel referred to above contains some data of value on susceptibility of corn varieties to mosaic. His presentation of the history of our knowledge of this disease in corn is very misleading. One of the present writers contributed the original paper on corn mosaic (4). This paper, which was based on careful observations and experiments established the type of disease, its infectious nature, its identity with sugar-cane mosaic, and the natural agents of transmission. Within its pages ample credit was given to two previous investigators for observation of a possibly similar condition in corn. Their observations were of a very indefinite nature and were summed up in the following words by the only one of the two who published on it (16):

The trouble was ascribed to various causes by different people, *but it appears to be the same baffling general condition observed in Hawaii rather than any specific disease.*<sup>8</sup>

On the basis of this candid admission and other less reliable evidence Kunkel, perhaps unintentionally, leads the reader to believe that the disease was well known and well understood prior to the present writers' contribution.

#### FIELD OBSERVATIONS ON SORGHUM AND PEARL-MILLET MOSAIC

Although mosaic on sorghum was early noted by one of the writers in experiments at Washington (2) and afterwards proved to be identical with sugar-cane mosaic (3), it was not observed in the field until the summer of 1920. At that time many fields of sweet sorghum were noticed to be naturally infected in the vicinity of Cairo, Ga. All such fields were within half a mile of affected sugar cane. No accurate data were obtained on injury to sorghum, but the leaf symptoms were strongly marked, the nondevelopment of chlorophyll being about the same as in severely injured sugar cane, and the plants were decidedly stunted. It was noticed that when plants were infected soon after germinating, the injury was far greater than in the case of late infection. A few notes were taken on varieties affected and on the percentage of infected plants in various fields.

The variety Honey appears to be especially susceptible. Several fields of this variety, known locally as Sugar Drip, were observed to contain 6

<sup>8</sup> The italics are ours.

to 30 per cent of diseased plants. Fields of the so-called Texas-Ribbon variety (probably Gooseneck) contained from 0.5 to 15 per cent of affected plants, or uniformly less than the first-named sort under the same conditions. No infection was observed in a field of Early Amber, but this variety has proved to be susceptible under greenhouse conditions at Washington. A number of grain-sorghum varieties recently introduced from Africa were planted in a greenhouse at Washington exposed to natural infection, but they have showed no signs of the disease. About 50 large stools of mosaic sugar cane were present in this house, and *Aphis maidis* was abundant.

During the summer of 1921 a patch of pearl millet (*Pennisetum glaucum*), sold under the name Georgia cat-tail millet, was planted for soiling purposes at the Sirup Experiment Station, Cairo, Ga. On July 15 this planting was noticed to be severely attacked by mosaic. More than 50 per cent of the plants were affected and were noticeably smaller than healthy plants. This species also had been previously proved to be susceptible to grass mosaic under controlled conditions at Washington. (See Table II.)

#### FIELD OBSERVATIONS ON MOSAIC OF WILD GRASSES

Collections of wild grasses affected with mosaic have been made in Louisiana, Georgia, and Florida. In all cases the plants were found in or near affected sugar-cane fields. That the mosaic appearing naturally in the field in these species is identical with sugar-cane mosaic has been verified experimentally under controlled conditions. (See Tables I and II.) A list of mosaic grasses collected with dates and localities is given in Table VIII.

TABLE VIII.—Collections of wild grasses affected with mosaic

Species and locality.	Date of collection	Species and locality.	Date of collection.
<i>Syntherisma sanguinalis</i> :		<i>Paspalum bosceanum</i> :	
Cairo, Ga. . . . .	Sept. 11, 1919	Cairo, Ga. . . . .	Sept. 11, 1919
New Orleans, La. . . . .	Oct. 15, 1919	Marianna, Fla. . . . .	Aug. 16, 1920
Do . . . . .	Sept. 6, 1920	Cairo, Ga. . . . .	July 27, 1920
Chattahoochee, Fla. . . . .	Sept. 1, 1920	Reno, Ga. . . . .	July 29, 1920
Plaquemine, La. . . . .	Aug. 21, 1920	<i>Chaetochloa magna</i> :	
Marianna, Fla. . . . .	Aug. 14, 1920	Dade County, Fla. . . . .	Dec. 11, 1920
Reno, Ga. . . . .	July 31, 1920	<i>Brachiaria platyphylla</i> :	
		New Orleans, La. . . . .	Aug. 11, 1920
		Do. . . . .	Sept. 6, 1920

No complete record of individual observations on mosaic of wild grasses has been kept. The foregoing list is sufficient to show that mosaic of these species is common near affected sugar cane under natural conditions.

#### EXPERIMENTS ON SEED TRANSMISSION OF GRASS MOSAIC

It seems appropriate to include in the present paper a brief account of experiments to determine whether the disease is transmitted by means of seeds, because of the relation of such transmission to the infection of one species with inoculum from a different species. In regions where the vegetative parts of annual grasses are completely killed during winter,

the virus would be more likely to persist in seeds, if it survives at all, than in any other state. The possibility of its being carried over in the bodies of insect vectors is rather remote, since there is no evidence that such insects function as intermediate hosts, but rather as mechanical carriers, and as such they would not furnish the special conditions necessary for long-continued survival of the virus. This view is supported by our results with artificial inoculations, which proved that development of the virus within the body of an insect is not necessary. We have fairly conclusive proof that the virus does not survive in plant trash or soil, even in tropical countries. Our evidence that the virus is not carried over in seeds (2) has been strengthened by the results of subsequent experiments with corn, sorghum, and wild-grass seeds from mosaic parents, all of which gave rise to healthy plants. The results are shown in Table IX.

TABLE IX.—Experiments to determine the transmission of disease by means of seeds from mosaic corn, sorghum, and wild grasses

Species.	Date planted	Number germinated.	Results.	
			Date examined.	Condition of seedlings.
<i>Zea mays</i> var. White Creole	Nov 30, 1920	44	Jan. 25, 1921	All healthy.
<i>Zea mays</i> var. U. S. Selection No. 182.	... do	81	do	Do.
<i>Zea mays</i> var. White Creole	Dec. 15, 1920	100	Feb. 15, 1921	Do.
<i>Zea mays</i> var. U. S. Selection No. 182.	do	90	do	Do.
<i>Holcus sorghum</i> var. Honey	Sept 15, 1919	181	Nov. 30, 1919	Do.
Do.....	Nov 30, 1920	94	Jan. 26, 1921	Do.
<i>Holcus sorghum</i> var. Goose-neck.	.... do	75	do	Do.
<i>Paspalum bosciannum</i> .....	Jan. 20, 1921	47	Mar 12, 1921	Do.
<i>Brachiaria platyphylla</i>	do	52	do	Do.
<i>Syntherisma sanguinalis</i> ..	do	42	do	Do.

Observations by Vander Stok (14), Kobus (11), and Wilbrink and Ledeboer (17) in Java indicate that sugar-cane seedlings from mosaic parents are healthy at the start and remain so unless infected from outside sources in the usual way. All available evidence, therefore, points to the conclusion that in this disease, as in tobacco mosaic, the virus is not transmitted from generation to generation by means of seeds.

#### SUMMARY

(1) Thirteen species of grasses have been proved by inoculation experiments to be susceptible to the disease known as sugar-cane mosaic or, more properly, grass mosaic.

(2) Certain varieties of sugar cane belonging to the slender North India type, formerly regarded as immune, have proved susceptible to mosaic.

(3) Data on the yield of 17 varieties of southern field corn show a decrease in weight of 0.4 to 50.6 per cent, due to mosaic.

(4) Field observations indicate that natural infection of sorghum, pearl millet, crab-grass, bull-grass, *Chaetochloa magna*, and *Brachiaria platyphylla* is widespread near affected cane in the sugar-cane belt.

(5) All species tested for seed transmission of mosaic gave negative results.

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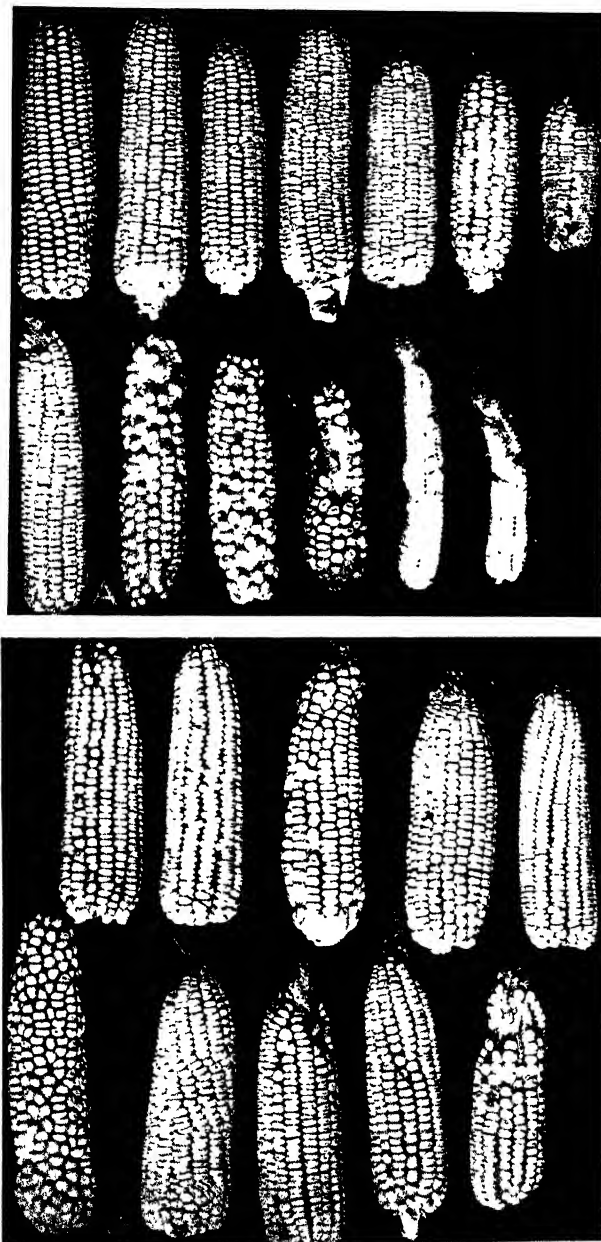


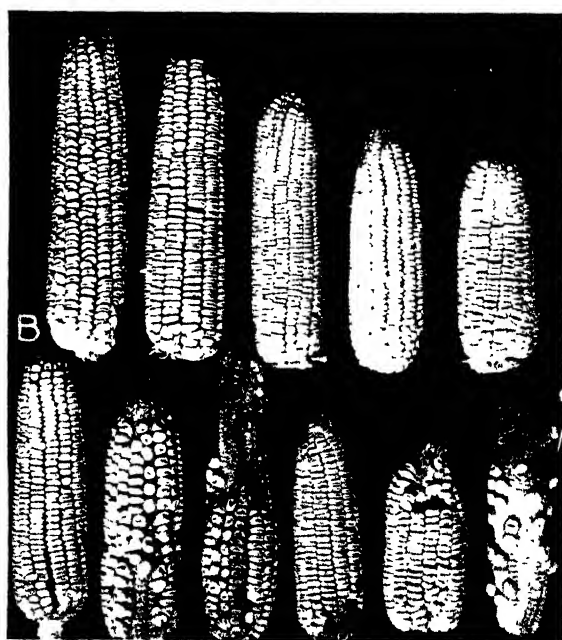
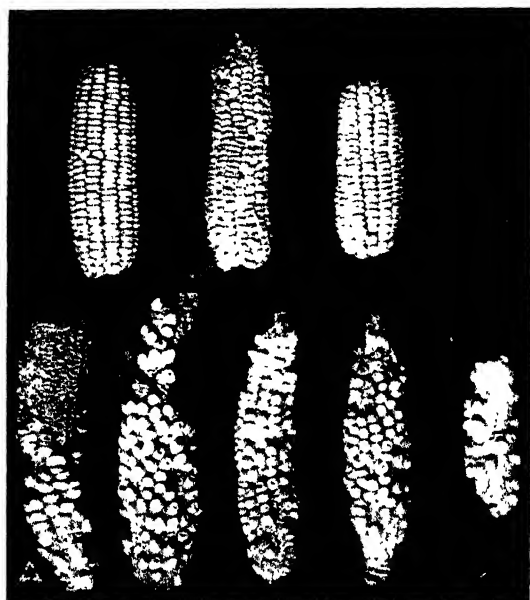


PLATE 1

A.—Ears of healthy (upper row) and mosaic (lower row) corn of Arlington Prolific variety.

B.—Ears of healthy (upper row) and mosaic (lower row) Laguna corn, a resistant variety.





**PLATE 2**

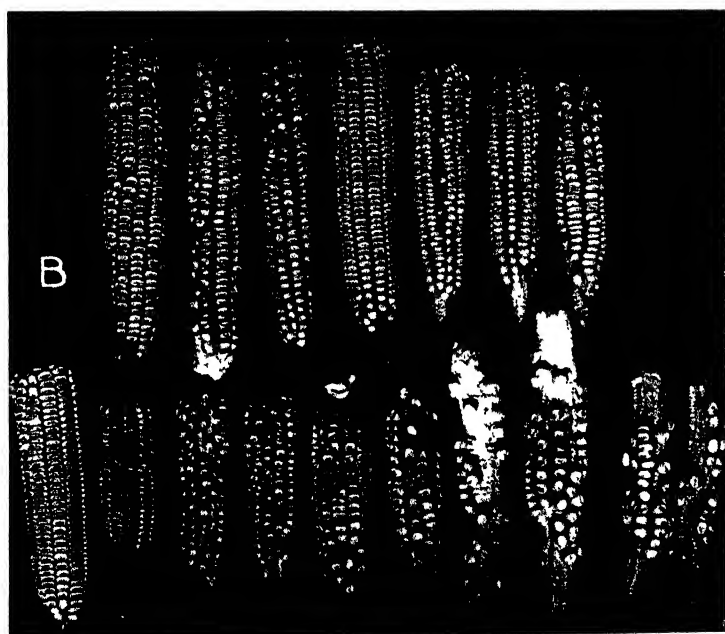
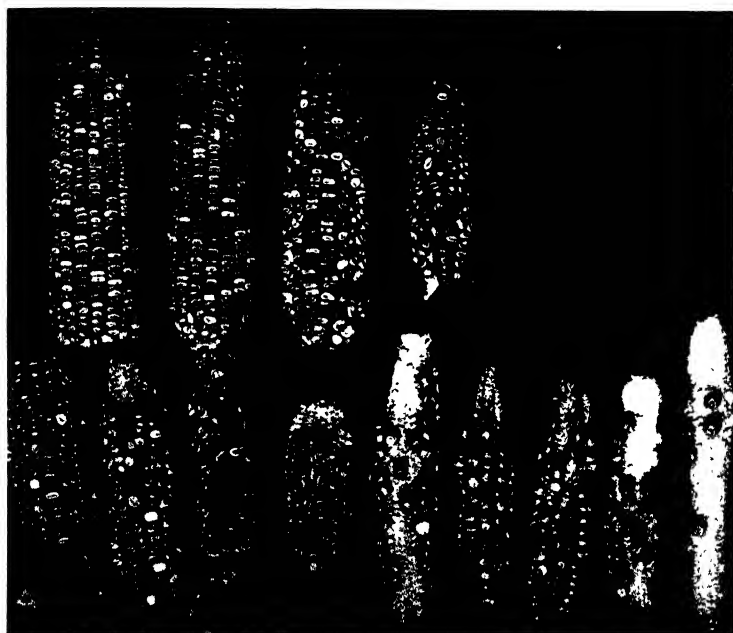
**A.—Ears of healthy (upper row) and mosaic (lower row) corn of U. S. Selection No. 119.**

**B.—Ears of healthy (upper row) and mosaic (lower row) corn of U. S. Selection No. 170.**

PLATE 3

A.—Ears of healthy (upper row) and mosaic (lower row) corn of Millpond Prolific variety.

B.—Ears of healthy (upper row) and mosaic (lower row) corn of U. S. Selection No. 165.



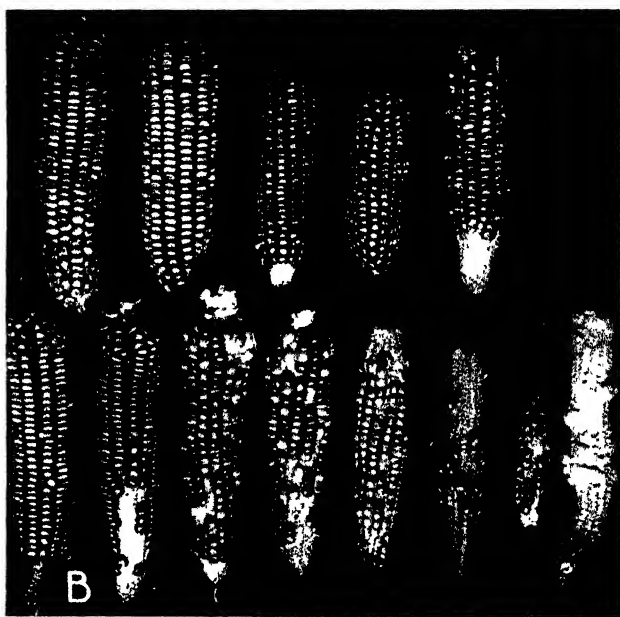
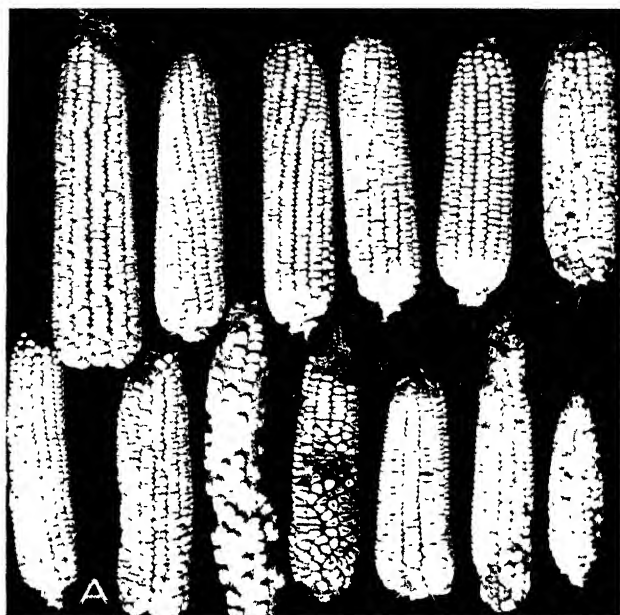


PLATE 4

A.—Ears of healthy (upper row) and mosaic (lower row) corn of Brazos variety.

B.—Ears of healthy (upper row) and mosaic (lower row) corn of Cuban Yellow variety.





# PROTEIN SYNTHESIS BY AZOTOBACTER<sup>1</sup>

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The metabolic process of protein synthesis is common to all microorganisms. The rate, form, and total quantity of protein synthesized, however, as well as the type of food substances available for synthesis, varies widely.

An idea of the relative protein content of microorganisms can be obtained from the results recorded by various investigators; but the multiplicity of the methods employed in the cultivation, collection, and analysis of microbial cells makes a comparative study difficult. A high nitrogen content for most bacteria is reported. If all of this nitrogen be considered as protein nitrogen, the protein content of such cells is large. Wheeler (8, p. 65),<sup>2</sup> whose results seem to be comparative for such analyses, reports a nitrogen content varying from 5.964 per cent to 11.765 per cent for 12 bacteria examined. The nitrogen content of yeasts is probably similar to that of bacteria. Nicolle and Alilaire (5) report a nitrogen content of 10.0 per cent for a Froberg yeast.

The high protein content of yeasts, their rapidity of growth, and their practical method of cultivation justify their wide exploitation as a food product within recent years. The value of such yeast food as a protein concentrate appears to be firmly established. During the recent war the demand for this yeast concentrate in Germany far exceeded the supply.

Interest in the yeast industry has been accentuated with the development of the present vitamin theory. The high content of water-soluble B vitamin in yeast cells has apparently opened a new field for its use.

Several factors tend to influence the practicability of utilizing microorganisms as a source of protein. Mechanically, the problem is dependent upon the development of practical methods for securing a maximum degree of growth, as well as means for the collection and care of the product. Economically, the yield, rate of growth, and the food requirements of the organism are important considerations. The substances used as food for the organism should be in the nature of waste products, and to justify the process the synthesized product must have a greater value than the substances used in its production. Finally, if the industry is to be of value, the product must have a nutritional value.

The high protein content of the *Azotobacter* cell and its relatively simple food requirements suggested the possibility of utilizing it as a means for synthesizing a protein which could be used either as a stock food or as a fertilizer. The employment of this organism for such a purpose appeared to offer some important advantages on account of its nitrogen-assimilating ability. This would necessitate the use of a solution having a carbohydrate only as the important constituent.

On the other hand, the apparent slow development of *Azotobacter* seemed to present a serious difficulty. However, it has been demonstrated

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 274.

that a prompt and heavy growth of *Azotobacter* can be secured by a vigorous aeration of a liquid culture in a manner similar to that used in stimulating yeast growth.

The experimental data which follow are merely a report of progress in an investigation having for its object the utilization of waste products by *Azotobacter* for protein synthesis.

#### METHOD OF CULTIVATION

A rapid and vigorous growth of *Azotobacter* was produced by passing a current of air continually through a culture solution. The method used was identical with that described in a previous publication (14).

Aeration was accomplished by attaching the culture flask equipped with Folin's aerating tubes to a vacuum system. Contamination and evaporation of the culture were reduced to a minimum by filtering the air through a sterile cotton filter and two or three flasks of sterile water. The air was thus filtered through cotton and rinsed in water before entering the culture flasks.

Several flasks of culture media could be easily aerated at the same time. Usually a cotton filter and a wash bottle were placed between the culture flasks. Little trouble occurred from contamination. A good grade of rubber tubing and tight fitting connections are a necessity, however. Flasks containing from 200 cc. to 2,500 cc. of medium were used. The quantity of air passed through the cultures was not measured, but a vigorous bubbling of the liquid was maintained continuously.

#### AZOTOBACTER CULTURES

Cultures of *Azotobacter* were isolated from samples of soil received from various parts of Kansas, Colorado, California, Iowa, and Mississippi. Flasks containing dextrose-Ashby or mannite-Ashby medium were inoculated with the soil samples. Upon the formation of the characteristic surface growth, Ashby agar plates were streaked. Dextrose-Ashby agar slants were inoculated from well-isolated colonies and repeated streakings of the cultures upon Ashby agar plates were made. A large number of the cultures were streaked consecutively from 1 to 12 generations. The utmost care was exercised in attempting to obtain and maintain pure cultures.

The inoculum used for seeding the medium was prepared by adding a portion of the emulsion from a young dextrose-Ashby culture to a flask containing dextrose-Ashby broth. This was aerated for two to four days. The quantity of this starter usually used for inoculating the experimental medium was 0.5 per cent to 1.0 per cent of the medium seeded. In all cases a morphological examination of the starter was made as a test for purity before use. The temperature for incubation was 30° C.

#### MEDIUM

The medium used in the following experiments, unless otherwise noted, was made from the following formula:

	Gm.
Tap water.....	1,000.0
K <sub>2</sub> HP O <sub>4</sub> .....	.5
Mg So <sub>4</sub> .....	.2
NaCl.....	.2
Dextrose.....	10.0

The reaction of this solution was readjusted to a  $P_H$  of 7.0 to 7.4, filtered if necessary. The required quantities were placed in flasks and sterilized in the autoclav at 20 pounds pressure for 30 minutes.

#### METHODS OF ANALYSIS

Total nitrogen determinations and sugar analysis of the *Azotobacter* cultures were made at frequent intervals.

In all cases the total nitrogen was determined by the usual standard methods. Unless otherwise noted, 50 cc. portions of the medium in duplicate were used. The figures referred to in the tables denote the average of the duplicate analyses. The net gain in nitrogen is recorded in all cases, unless otherwise stated, as milligrams of nitrogen in each 100 cc. of medium. In other words, the figures refer to the quantity of nitrogen fixed per gram of dextrose, since each 100 cc. of medium contains this quantity of sugar.

Sugar determinations were made according to the method proposed by Shaffer (9). The copper resulting from the reduced Fehlings was determined by colorimeter readings. Duplicate readings were made each time, and the average of these was recorded. As a general rule, 50 cc. of the medium were used. This was diluted to 100 cc., and 20 cc. of this filtrate were used for reduction. The figures given, unless otherwise stated, refer to grains of dextrose per 100 cc. of medium.

#### COMPOSITION OF THE AZOTOBACTER CELL

The composition of the *Azotobacter* cell has been determined by several investigators. Gerlach and Vogel (1) report a protein content of 80 per cent.

Lipman (2) reports the nitrogenous composition of the *Azotobacter* membrane as 10.45 per cent total nitrogen, 6.39 per cent nonbasic nitrogen, 2.76 per cent basic nitrogen, and 0.98 per cent ammonia nitrogen. He observed that lead acetate precipitated practically all of this nitrogen in young or old cultures, while phototungstic or tannic acid would not precipitate nearly as much. He believed that in young cultures this nitrogen substance is in a soluble form and not precipitated by phosphotungstic acid, but as the culture grows older this soluble nitrogenous material is converted into an insoluble and more complex protein.

Stoklasa (4) cultivated *Azotobacter* in liquid cultures. The growth was collected on a filter and washed. The nitrogen content of the washed cells was 11.3 per cent.

Hoffmann and Hammer (6) studied the composition of *Azotobacter* and report analyses much lower in protein. The organism was grown on Ashby agar plates. The growth was scraped off, washed, and dried. The maximum protein content recorded was 17.75 per cent. They suggest that the wide difference in their results, as compared with those of other investigators, was due to the jellylike material which in liquid cultures is filtered out of solution. In their method this material is included in the analysis. This increases the total residue, and since this substance is thought to be carbohydrate, the total percentage of nitrogen is decreased. The phosphorus content ( $P_2O_5$ ) varied from 2.51 per cent to 2.97 per cent. Increasing with the age of the culture. Stoklasa (4) reports the  $P_2O_5$  content to be 4.93 per cent.

Omeliansky (7) grew *Azotobacter* on dextrose mineral salt agar for six days at a temperature of 30° C. An analysis of this growth is

reported as 6.63 per cent moisture, 4.16 per cent ash, and 12.92 per cent protein. This protein he considers similar to other plant proteins.

While the exact method of cultivation is not reported by all investigators, it appears in general that the nitrogen content of *Azotobacter* will vary according to the method of cultivation. The nitrogen of the growth scraped off Ashby agar media is reported much lower than in the residue filtered from the liquid cultures.

The composition of seven cultures of *Azotobacter* cultivated on dextrose-Ashby agar was determined in this laboratory. The growth at the end of two to four days incubation at 30° C. was scraped off the surface of the agar, air dried, and analyzed for the total nitrogen and moisture content. The results are given in Table I.

TABLE I.—Nitrogen content of *Azotobacter* cells grown on Ashby agar

Culture No.	Total nitrogen.	Moisture.
	Per cent.	Per cent.
12 B.....	3.48	9.12
232.....	3.78	8.55
6 A.....	3.78	10.00
5 B.....	3.09	6.89
1 B.....	3.74	8.10
216.....	4.55	4.69
10 B.....	3.74	9.48
Average .....	3.73	8.12

The percentage of total nitrogen noted in the seven cultures was uniform, averaging 3.73 per cent for all cultures and ranging from 3.48 per cent to 4.55 per cent. If this nitrogen were calculated as protein, the average protein content of the cultures would be 23.31 per cent.

An analysis of a composite sample of these cultures gave the following results: Moisture 8.58 per cent; total nitrogen 3.55 per cent; albuminoid nitrogen 1.89 per cent; ash 12.99 per cent; phosphorus 0.57 per cent; and potassium 1.43 per cent. These figures indicate that only 53.1 per cent of the total nitrogen is protein. This gives a protein content of 11.81 per cent instead of 23.31 per cent, as calculated from the total nitrogen.

The chemical composition of the growth obtained from liquid cultures was determined for comparison. Culture No. 232 was aerated from two to four days at 30° C. in the dextrose-Ashby medium. The growth of cells was obtained by centrifuging in a Sharples laboratory supercentrifuge. A composite sample taken from the growth of several culture flasks was used for analysis. The composition was: Moisture 2.67 per cent; total nitrogen 5.15 per cent; albuminoid nitrogen 4.89 per cent; ash 4.62 per cent; phosphorus 0.24 per cent; and potassium 1.2 per cent.

A comparison of this analysis with that obtained from the growth on Ashby agar reveals considerable difference. In the first place, a higher total nitrogen content is observed in the growth from the liquid culture. The most marked effect, however, is the high albuminoid content of the liquid culture, it being 94.9 per cent of the total nitrogen. This gives a protein content of 30.56 per cent, as compared with 11.81 per cent for the growth obtained from Ashby agar. The liquid culture produced a growth with a lower ash content than did the culture on agar.

## SOURCES OF ENERGY

There is a wide variety of substances available as energy for *Azotobacter* which differ greatly in their value as sources of energy. Not only is there a difference in the value of the sources of energy for nitrogen fixation, but the efficiency of the same material for azofication varies, according to different investigators. This is illustrated in the work of Löhnis and Pillai (3), Hoffmann and Hammer (6), and Mockenridge (10). There appears to be, however, a rather uniform opinion that a mannite solution will furnish energy for the fixation of the largest quantity of nitrogen. As a result, mannite is the carbohydrate employed almost universally in azofication experiments.

As a preliminary study, the comparative value of various substances as sources of energy for *Azotobacter* was determined. One per cent of the test material was substituted for mannite in Ashby's medium containing calcium carbonate. Flasks of each medium were inoculated with a pure culture of *Azotobacter* and aerated at 30° C. for six days. The average quantity of nitrogen fixed per gram of test substance for the two cultures studied were as follows:

	Mgm.
Potassium acetate . . . . .	8.0
Dextrose . . . . .	7.8
Saccharose . . . . .	7.7
Mannite . . . . .	7.2
Molasses . . . . .	3.0
Lactose . . . . .	2.4

Dextrose has been preferred in this laboratory as a source of energy for studying the nitrogen-fixing ability of different cultures and their rate of fermentation. Quantitative determination can be easily made and, as shown by these experiments with aerated cultures, it is an efficient source of energy for azofication.

While no special endeavor has been made to study the nitrogen-fixing ability of different *Azotobacter* cultures with the object of classifying them on such a basis, over 20 strains have been studied. The nitrogen fixation for these cultures grown in dextrose medium has varied from 7.20 mgm. to 18.72 mgm. per gram of dextrose. Many of the same cultures have been repeatedly used for the past two years, and the variations in the quantity of nitrogen fixed at different times by the same cultures is as much as the difference between various cultures. This variation, or at least a large portion, may be attributed to the different intensities of aeration to which the cultures were submitted. The aim was to aerate all cultures alike, but such is impossible unless the aeration is mechanically controlled. This suggests that the optimum quantity of air necessary for the maximum growth of *Azotobacter* should be determined.

## YIELD OF AZOTOBACTER GROWTH

Cultures of *Azotobacter* cultivated by vigorous aeration in a liquid medium will exhibit a vigorous growth within two to four days. The sugar is rapidly consumed, and a corresponding increase in the nitrogen content of the liquid is noted. If the culture produces pigment, coloration of the medium is observed. In some cultures this coloration is black; in others brown. In those cultures which fail to produce pigment the solution becomes thick and milky in appearance. Pure cultures maintain an alkaline reaction in the absence of calcium carbonate in the medium throughout growth and emit a rather pleasant odor.

A rather interesting phenomenon was often noted in these liquid cultures when treated with a lead-acetate solution. If the heavy white membranous precipitate which forms is collected on a filter, it gradually darkens and within several minutes is black. The reaction resembles an enzymatic process. No attempt was made to ascertain whether or not this process is connected with the characteristic pigment production in the living cells.

The yield, or amount of growth, was determined by centrifuging 50 cc. of the culture until the supernatant fluid was clear. This was decanted off, care being taken not to disturb the precipitate cells. The centrifuge tube containing this precipitated growth was placed in a drying oven and desiccated for eight hours at 75° to 80° C. to a constant weight. Duplicate determinations of each culture were made. The yield is recorded as a percentage of sugar originally present in the medium.

A daily comparison of the nitrogen assimilation, dextrose fermentation, and the yield of three cultures of *Azotobacter* is summarized in Table II. Four flasks containing 250 cc. of dextrose medium were used for each culture. All were aerated vigorously at a temperature of 30° C. One flask of each culture was removed daily for analysis. The medium contained 1 gm. of dextrose per 100 cc.

TABLE II.—Yield rate of dextrose fermentation and nitrogen fixation by *Azotobacter*

	1 day.				2 days.			
	Culture No.			Average.	Culture No.			Average.
	10 B	19	19-399		10 B	19	19-399	
Nitrogen per 100 cc. (mgm.).....	1. 82	4. 31	5. 60	3. 91	8. 77	8. 21	10. 20	9. 06
Dextrose per 100 cc. (gm.).....		. 49	. 67	.....	. 478	0	. 06	.....
Yield per 50 cc. (gm.).	{ . 0920	. 0743	. 0319	.....	. 0901	. 1939	. 1281	.....
Yield (per cent).....	{ . 0750	. 0717	. 0154	.....	. 0784	. 1741	. 1198	.....
	16. 70	14. 50	5. 73	12. 31	16. 85	34. 80	24. 79	28. 81
	3 days.				4 days.			
	Culture No.			Average.	Culture No.			Average.
	10 B	19	19-399		10 B	19	19-399	
Nitrogen per 100 cc. (mgm.).....	9. 36	13. 72	12. 50	11. 86	12. 0	14. 43	12. 90	13. 11
Dextrose per 100 cc. (gm.).....	. 05	0	0	.....	0	0	0	.....
Yield per 50 cc. (gm.).	{ . 0841	. 1487	. 1234	.....	. 1043	. 1317	. 1017	.....
Yield (per cent).....	{ . 0841	. 1595	.....	.....	. 0889	. 1528	. 1149	.....
	16. 82	30. 82	24. 68	24. 10	19. 32	28. 45	21. 66	23. 14

There occurs a gradual increase in the quantity of nitrogen fixed. The average quantity fixed for the three cultures was for the first day 3.91 mgm., for the second day 9.06 mgm., for the third day 11.86 mgm., and for the fourth day 13.11 mgm., per 100 cc. of medium. The characteristic rapid disappearance of dextrose occurred. It was practically all consumed by the three cultures within two days.

The average yield of the three cultures for four consecutive days was as follows: 12.31 per cent, 28.81 per cent, 24.10 per cent, and 23.14 per cent of the dextrose originally present in the medium.

The effect of different quantities of dextrose upon nitrogen fixation and the yield was determined for media containing 0.5 per cent and 1.0 per cent dextrose. Flasks containing 250 cc. of medium were seeded with *Azotobacter* and aerated for four days at 30°C. Total nitrogen and yield determinations were made at the end of the incubation period. The results for the two cultures studied are recorded in Table III.

TABLE III.—Effect of varying quantities of dextrose upon yield and nitrogen fixation by *Azotobacter*

Culture No.	0.5 per cent dextrose.			1 per cent dextrose.		
	Weight.	Yield.	Nitrogen per 100 cc.	Weight.	Yield.	Nitrogen per 100 cc.
	Gm.	Per cent	Mgm.	Gm.	Per cent	Mgm.
19.....	0.0413 .0437	17.0	9.88	0.1014 .1153	21.66	16.38
232.....	.0322 .0357	13.58	7.57	.1143 .1282	24.24	16.65
Average.....	.. . . .	15.29	8.72	.....	22.95	16.57

The largest yield for the quantity of dextrose used was obtained from the medium containing 1 per cent dextrose. The average yield for the two cultures was 22.95 per cent for this medium as compared with 15.29 per cent for the cultures cultivated in the 0.5 per cent dextrose medium. In the 1.0 per cent solution the cultures fixed on an average 16.57 mgm. of nitrogen per gram of sugar; and in the media containing 0.5 per cent dextrose, an average of 8.72 mgm. of nitrogen per half gram of dextrose. In other words, the rate of fixation was practically the same in both solutions.

A similar experiment was made with media containing approximately 0.5 per cent, 1.0 per cent, and 1.5 per cent of dextrose, respectively. The yield with each percentage of dextrose used, the quantity of nitrogen fixed, and the rate of dextrose fermentation were determined every two days for a period of six days. The cultures were vigorously aerated at 30°C. The dextrose content of the control media by analysis was 0.60 per cent, 1.1 per cent, and 1.5 per cent, respectively. The results are summarized in Table IV. The percentage of yield is based upon the quantity of dextrose originally present in the media.

TABLE IV.—Comparison of various quantities of dextrose in culture No. 19 upon yield, nitrogen fixation, and dextrose fermentation.

	0.6 per cent dextrose.			1.1 per cent dextrose.			1.5 per cent dextrose.		
	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
Weight (gm.) . . . . .	0.0354 .0328	0.0399 .....	0.0692 .0669	0.0766 .0574	0.1506 .1280	0.1245 .1317	0.1190 .1354	0.2383 .2713	0.2036 .1928
Yield (per cent) . . . . .	11.3	13.3	22.6	12.1	25.3	23.3	16.9	33.9	27.0
Dextrose (gm.) . . . . .	.20	Trace	0	.68	Trace	0	.98	Trace	0
Nitrogen (mgm.) . . . . .	6.37	9.75	9.70	7.41	17.29	18.72	9.1	17.68	26.0



The results show that the yield increases with an increase in the concentration of dextrose in the media. An average of the three periodical analyses for each medium gives a yield of 15.7 per cent for the 0.6 per cent dextrose solution, 20.2 per cent for the 1.1 per cent solution, and 25.9 per cent for the 1.5 per cent solution.

The total nitrogen content of each medium at the completion of the experiment reveals the fact that nitrogen fixation occurred at the rate of 16.1 mgm. of nitrogen per gram of dextrose in the 0.6 per cent dextrose solution, 17.0 mgm. of nitrogen per gram of dextrose in the 1.1 per cent dextrose solution and 17.3 mgm. of nitrogen per gram of dextrose in the 1.5 per cent dextrose medium.

The dextrose disappeared rapidly from all solutions, being entirely consumed by the sixth day.

#### UTILIZATION OF MOLASSES

The waste molasses from a sugar factory should offer an available source of energy for *Azotobacter*. Hence an experiment was conducted in which molasses was substituted for dextrose in the medium, and the azofication ability of cultures was determined in this medium. The medium contained 1 per cent molasses, or by analysis 0.51 per cent of invert sugar. The quantity of nitrogen fixed per 100 cc. of medium for five cultures aerated for six days was as follows:

	Mgm.
1 B.....	1.5
2 B.....	3.3
5 B.....	3.3
12 B.....	3.4
232.....	3.6

The results show an average net gain of 3.03 mgm. of nitrogen per gram of molasses. A gram of molasses represents only 0.51 per cent of invert sugar, giving, therefore, a rate of fixation of 6.06 mgm. of nitrogen per gram of invert sugar.

To determine the yield from molasses, 250 cc. of the 1 per cent molasses medium was inoculated with culture No. 19 and aerated for four days at 30° C. The medium was analyzed for sugar, total nitrogen, and yield. The results are given in Table V.

TABLE V.—Yield, nitrogen fixed, and sugar fermented from molasses

Sugar per 100 cc.		Nitrogen per 100 cc.			Weight per 50 cc.			Yield.
Control.	Culture.	Gross.	Control.	Net.	Gross.	Control.	Net.	
Gm.		Mgm.	Mgm.	Mgm.	Gm.	Gm.	Gm.	Per cent.
0.50	Trace.	10.53	8.15	2.38	{ 0.0799 0.0935	{ 0.0063 0.0149	{ 0.0761	30.44

The total nitrogen content of different samples of molasses has varied from about 6 to 12 mgm. per gram of molasses. This nitrogen appears to be in a soluble form, since lead acetate fails to precipitate it. On the other hand, if a lead acetate solution is added to an *Azotobacter* culture cultivated in this molasses medium, a heavy membranous precipitate forms. An experiment, therefore, was arranged to ascertain whether

this nitrogen had been synthesized into a more complex nitrogenous substance by the growth of *Azotobacter*.

Eight flasks containing 250 cc. of molasses medium were inoculated, four with culture No. 19 and four with culture No. 232, and aerated thoroughly for eight days.

The contents of the four flasks of each culture were mixed, and total nitrogen determinations were made. The remaining portion was precipitated with a lead-acetate solution and filtered. This filtrate was perfectly clear. Total nitrogen determinations were likewise made on this filtrate. The control medium was treated in a similar manner.

The average results for all duplicate determinations are as follows:

Control.....	{Medium 6.71 mgm. nitrogen per 100 cc. Filtrate, 6.40 mgm. nitrogen per 100 cc.
<i>Azotobacter</i> No. 19.....	{Culture 9.52 mgm. nitrogen per 100 cc. Filtrate 0.8 mgm. nitrogen per 100 cc.
<i>Azotobacter</i> No. 232.....	{Culture 10.30 mgm. nitrogen per 100 cc. Filtrate 0 mgm. nitrogen per 100 cc.

From these results it is evident that *Azotobacter* is capable of synthesizing the nitrogen present in the molasses into more complex substances as well as assimilating nitrogen from the air.

#### UTILIZATION OF STRAW

Pringsheim and Lichtenstein (13) report an investigation the purpose of which was to enrich straw concentrate with protein by means of fungi. Hydrolyzed straw was spread out in thin layers and inoculated with an *Aspergillus*. The protein content of the straw increased from 0.9 per cent to 8.0 per cent within a week. Digestion experiments with the food proved satisfactory.

The utilization of vegetable tissues as available sources of energy for *Azotobacter* has been reported by Murray (11) and Hutchinson (12).

Experiments were undertaken in the present case with a view to increasing the protein content of wheat straw by *Azotobacter*. To each 200 cc. of a dextrose, and also a molasses medium, there was added 1 per cent of a good grade of wheat straw which had been finely ground in a mill. This was seeded with *Azotobacter* cultures and aerated for varying lengths of time. Total nitrogen determinations were made in duplicate on the entire contents of the culture flasks. To note the effect of straw in the medium, similar determinations were made on cultures containing no straw.

The results are recorded in Table VI for three cultures aerated in the dextrose solution and in the straw dextrose medium for four days.

TABLE VI.—Effect of straw on azofication

Culture No.	Dextrose-Ashby medium (200 cc.)			Dextrose-Ashby and straw medium (200 cc.)		
	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.
	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.
1 B.....	1.4	7.80	6.4	9.4	20.01	106.1
10 B.....	1.4	8.08	6.68	9.4	19.86	10.46
232.....	1.4	9.97	8.57	9.4	22.36	12.96

The net gain of nitrogen fixed by all the cultures in the straw solution exceeded the net gain in the dextrose by an average of 4.13 mgm.

The experiment was repeated, and a daily analysis of the cultures was made. The cultures in this case were aerated for four days. The data are tabulated in Table VII.

TABLE VII.—*Effect of straw on azofication*

Culture No.	Medium	Nitrogen in control.	1 day.		2 days.		3 days.		4 days.	
			Gross nitrogen	Net nitrogen	Gross nitrogen	Net nitrogen	Gross nitrogen	Net nitrogen	Gross nitrogen	Net nitrogen
10 B.	D. A. 200 cc. <sup>1</sup>	Mgm. 1.0	Mgm. 4.55	Mgm. 3.55	Mgm. 18.46	Mgm. 17.46	Mgm. 19.20	Mgm. 18.20	Mgm. 24.96	Mgm. 23.96
10 B.	D. A. S. 200 cc. <sup>2</sup>	10.07	10.40	.33	20.93	9.87	30.29	20.22	29.44	19.37
19.	D. A. 200 cc. <sup>1</sup>	1.0	8.38	7.38	15.14	14.14	24.70	23.70	25.02	24.02
19...	D. A. S. 200 cc. <sup>2</sup>	10.07	13.78	3.71	25.02	14.95	39.32	29.25	42.83	32.76

<sup>1</sup> Dextrose-Ashby 200 cc.

<sup>2</sup> Dextrose-Ashby and straw 200 cc.

In general, the results are similar to those reported in the previous experiment. However, culture 10 B showed an approximate net gain of 2.0 mgm. at the end of the third day in the straw culture and a loss of over 4.0 mgm. of nitrogen on the fourth day. An average of the two cultures indicates a net gain of 4.15 mgm. for the straw medium for the entire period of the test.

As expected, a greater net gain of nitrogen was recorded for the dextrose solution than for the straw medium for the first two days. This indicates that the straw is not utilized until all of the greater part of the dextrose is consumed.

Calculating all the nitrogen in the wheat straw as protein nitrogen indicates a protein content of 3.14 per cent. In a similar manner, calculating the nitrogen in the straw solution as protein nitrogen indicates an average protein content of the straw from the two cultures of 11.29 per cent, thus giving a net gain of 8.15 per cent protein.

However, but little of this net gain of protein can be attributed to the influence of the straw, for an average net gain of only 2.07 mgm. of nitrogen per gram of straw was noted in the straw dextrose media.

A summary of both experiments indicates an average net gain of only 1.54 mgm. of nitrogen per gram of straw when added to dextrose medium.

In another experiment three cultures were aerated for four days in a molasses solution and three other cultures in a straw-molasses solution. The results are recorded in Table VIII.

TABLE VIII.—*Effect of straw and molasses on azofication*

Culture No.	Molasses medium (200 cc.).			Molasses and straw medium (200 cc.).		
	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.
	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.	Mgm.
1 B.	17.54	19.69	2.15	25.37	28.98	3.61
10 B.	17.54	21.50	3.96	25.37	33.88	8.51
232.	17.54	18.57	1.03	25.37	28.55	3.18

The data show a net gain of nitrogen in favor of the straw medium for all cultures. The average net gain of nitrogen for all cultures grown in the molasses solution was 2.38 mgm. and for those grown in the straw solution 5.10 mgm. This gives an average of net balance in favor of the straw media of 2.72 mgm.

Another experiment was conducted in which two flasks containing 200 cc. of straw-molasses solution were seeded with two *Azotobacter* cultures and aerated for three days. Ferric-sulphate solution was added to precipitate the protein in each culture flask. The entire contents of each flask were then placed upon a filter, and the residue was collected. This precipitate was desiccated and total nitrogen determinations were made. A control medium was treated in a similar manner. The results are presented in Table IX.

TABLE IX.—*Effect of straw and molasses on azofication*

Culture No.	Total nitrogen per gram of straw.	Net nitrogen per gram of straw.
	Mgm.	Mgm.
Control.....	11.18	.....
232.....	15.41	4.23
1 B.....	15.84	4.66

The experiment shows an average net gain of 4.44 mgm. of nitrogen per gram of straw, or 2.77 per cent protein.

## SUMMARY

(1) The protein content of *Azotobacter* growth obtained from a solid medium was found to be 11.81 per cent, while that collected from a liquid culture was 30.56 per cent.

(2) The yield of cells increased with the quantity of dextrose in the medium. An average yield of 15.7 per cent, 20.2 per cent, and 25.9 per cent of the sugar was obtained from medium containing 0.6 per cent, 1.1 per cent, and 1.5 per cent dextrose, respectively.

(3) The relative quantities of nitrogen fixed per gram of dextrose for the three different percentages of sugar were similar—namely, 16.1 mgm., 17.0 mgm., and 17.3 mgm., respectively.

(4) When molasses was used as a source of energy for *Azotobacter* development there was obtained a yield of cells equal to 30.44 per cent of the sugar in the molasses. This gives a higher percentage yield for the molasses sugar than for dextrose. If the moisture content of the molasses is considered, the percentage yield from the actual molasses exceeds the yield from dextrose.

(5) *Azotobacter* is able to convert the soluble nitrogenous substances present in molasses into more complex protein, as well as to utilize the molasses as a source of energy for the fixation of atmospheric nitrogen.

(6) The addition of straw to the dextrose or molasses medium did not cause any appreciable increase in the quantity of nitrogen fixed.

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## STUDIES ON THE TEMPERATURE OF INDIVIDUAL INSECTS, WITH SPECIAL REFERENCE TO THE HONEY BEE<sup>1</sup>

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### INTRODUCTION

More observations have been made on the temperature of the bee colony than on the individual bee. Phillips and Demuth (16)<sup>3</sup> concluded that bees, essentially cold-blooded animals, were capable of regulating their temperature when in a colony by increasing the temperature of the colony when the air temperature went down and lowering the temperature of the colony when the air temperature went up. Since Phillips and Demuth have shown that the colony, which is composed of a large number of individuals, acts very much as a warm-blooded animal does, the temperature of the individual must be of great importance. If this were not true, it would be difficult to understand how the colony of individuals could control its temperatures. For this reason and because of the wide variation in the results obtained by previous workers this problem was chosen for study.

In a review of the literature on this subject it was found that previous workers had based the results of their observations on a few individuals. The results included in this paper are based on the readings of over 1,000 bees. The methods and results of the previous workers are given in the following review of the literature.

### REVIEW OF LITERATURE

Hausmann (10) was the first to establish the temperature of an individual insect. He placed a *Sphinx convolvuli* together with a small thermometer in a glass receptacle, the air temperature of which was 17° R. (21.25° C.). After half an hour the temperature had risen to 19° R. (23.75° C.) and soon after fell to 17° R. Experiments with *Carabus hortensis* gave the same results.

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<sup>2</sup> The work on which this paper is based was done in the laboratories of the Division of Entomology of the University of Minnesota. To the members of the staff, and especially to Dr. R. N. Chapman, under whose immediate direction the study was conducted, acknowledgments are due for apparatus furnished and for constant helpful suggestions.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 286-287.

Davy (4) was the first to give the internal temperature of an insect. An incision was made in the body and the bulb of a very small mercurial thermometer was inserted. He obtained the following results:

	Air temperature.	Insect temperature.
	° C.	° C.
<i>Blatta orientalis</i> . . . . .	28.3	23.9
Do . . . . .	23.3	23.9
Gryllidae . . . . .	16.7	22.5
Vespidæ . . . . .	23.0	24.4
Do . . . . .	24.3	25.0
Lampyridæ . . . . .	22.8	23.0
Do . . . . .	26.6	25.8

Nobili and Melloni (15) were the first to use electric methods in determining the temperature of insects. They used the electromotive force developed when the junction of wires of different metals of a common circuit are at different temperatures. They used bismuth and antimony wires. One of the thermocouples was in contact with the insect body; the other couple was free. They made a series of experiments on the temperature of larvae, pupae, and adult butterflies. Their conclusion was that the temperature of the insect was higher than that of the surrounding air.

Mussehl (13) ascertained that single bees became motionless at 5° R (6.25° C.) while they did not suffer from cold in the colony with the temperature of the hive at -1° R. (-1.25° C.).

Newport (14) used a thermometer of small caliber with a cylindrical bulb about one-half inch in length. The thermometer was placed beneath the insect and was as completely covered by the abdomen of the insect as possible. A second thermometer which had been carefully compared with the first was placed at the same level with and a short distance from the first one. The temperature of the insect was taken on the exterior and was always lower than that of the interior. He stated that the internal temperature was seldom if ever more than a degree and a half or at the most two degrees above the external temperature. The following are some of his results showing the difference between the air temperature and the external temperature of the insect:

	Temperature of air.	Body temperature.	Temperature difference.
	°F.	°F.	°F.
<i>Bombus terrestris</i> . . . . .	66.9	73.4	6.5
Do . . . . .	66.9	76.2	9.3
Do . . . . .	66.9	73.4	6.5
Do . . . . .	69.4	76.2	6.8
Do . . . . .	68.0	77.5	9.5
<i>Bombus lapidarius</i> . . . . .	68.0	71.5	3.5

At the time of taking the foregoing readings the insects were in an excited condition.

Dutrochet (7) used a thermocouple made by soldering an iron and copper wire together. He placed one couple in the body of the insect and the other couple in the body of a dead insect of the same species or in paper. He fastened several bees to a hive by means of a thread and took temperature readings while the bees were very active. One couple was placed 5 mm deep in the body of the bee; the other couple was wrapped in paper to protect it from radiation. The air temperature was constant for four hours (19.2°C.). This experiment showed that the temperature of the insect was 0.18°C. lower than that of the surrounding air. The bees were next placed in a bell jar with the air dampened. They then had a temperature of 0.18°C. higher than the air temperature.

Experiments with *Bombus hortorum* were also performed. The thermocouple was not placed in the body but merely placed against it. The bee was then wrapped up in a piece of gauze which caused it to become very much excited. Under these conditions the temperature was 0.5°C. higher than that of the surrounding air. As the bee became quiet, its temperature was 0.03°C. lower than the air temperature. Dutrochet concluded that insects when active had a temperature higher than the surrounding air, when inactive a temperature that corresponded to that of the surrounding air.

Dzierzon (8) observed that bees became motionless at 5°R. (6.25°C.).

Dönhoff (5) placed 200 bees in a glass container the temperature of which was 22.5°C. In a short time the temperature had risen to 34.4°C. He also pressed an individual bee against a thermometer and found it to have a temperature of 15/16°C. higher than the air. He concluded that the difference in temperature between the bee and the surrounding air was greater when the air temperature was low and less when the air temperature was high.

Schönfeld (17) placed 100 bees in a water glass which was perfectly dry. A piece of old dried wax was attached to the cover of the glass in which there was a hole for the insertion of the thermometer. A small board was placed in the bottom of the glass on which the thermometer rested. The entire apparatus was placed on a cook stove. After 3 hours and 25 minutes the thermometer had risen from 10°R. (18.75°C.) to 31.5°R. (39.3°C.). All the bees, with the exception of 5, were found standing on the wax. One bee began to fan as the temperature reached 32.7°R. (40.8°C.) and at 36°R. (45.0°C.) 80 bees fell to the bottom of the glass and died. Sixteen bees were still living at 46°R. (57.5°C.), and when the cover was removed they flew out. The experiment was repeated and 2 bees withstood the temperature of 48.2°R. (60.2°C.) and flew away when the glass was opened.

Girard (9) carried on several experiments on insect temperature and found that the bumblebee had a lower temperature when in lack of honey. He concluded also that Hymenoptera had a higher temperature than the surrounding air but a significantly lower temperature than the Lepidoptera and Diptera.

Dönhoff (6) observed that bees soon died at -1.5°C. when placed in frozen ground.

Molin (12) found that bees became motionless at 5°R. (6.25°C.) and that at 7°R. (8.75°C.) they cleaned themselves and carried water. They left the hive and flew into the field at 12°R. (15°C.).

Koschewnikow (11) noted that bees kept at a temperature between 0° and 1°R. (0° to 1.25°C.) for 10½ hours lived. Other bees that were motionless for 30 hours at a temperature of -1°R. (-1.25°C.) also



survived. After the bees were kept at  $-2^{\circ}\text{R.}$  ( $-2.5^{\circ}\text{C.}$ ) for 50 minutes they died.

In his work on maximum temperature he concluded that:

1. There was a range of  $9^{\circ}\text{R.}$  ( $11.25^{\circ}\text{C.}$ ) in the fatal temperature of bees.

2. Dryness and dampness had no effect on bees in a high temperature.

3. Workers and drones became very much excited when the temperature was above  $30^{\circ}\text{R.}$  ( $37.5^{\circ}\text{C.}$ ).

4. The highest minimum fatal temperature at which the workers died was  $35^{\circ}\text{R.}$  ( $43.75^{\circ}\text{C.}$ ), while the drones died at  $30^{\circ}\text{R.}$  ( $37.5^{\circ}\text{C.}$ ).

5. The highest temperature which the bees could withstand was  $44^{\circ}\text{R.}$  ( $55^{\circ}\text{C.}$ ).

6. The workers immediately upon hatching showed a sensitiveness to heat, none surviving more than  $39^{\circ}\text{R.}$  ( $48.75^{\circ}\text{C.}$ ).

7. Still younger bees with the chitin less hard but apparently well formed were in contrast to the ones mentioned above because they were more capable of withstanding a higher temperature. They died at  $52^{\circ}$  to  $53^{\circ}\text{R.}$  ( $65^{\circ}\text{C.}$ ). The explanation of this phenomenon appears to lie in the fact that their bodies contained and evaporated considerable moisture and that they did not move.

8. Upon bringing the bees immediately into high temperature Koschewnikow observed that the period which passes between the time of inserting the bees and their death became shorter with increases in temperature.

In the following review of the experiment no statement was made as to whether or not the figures given were the actual internal temperatures of the insects.

Period of exposure	Temperature to which bees were exposed.	Temperature of bees at death.
	$^{\circ}\text{R.}$	$^{\circ}\text{R.}$
2 minutes	44	45
2 minutes 10 seconds	45	46
2 minutes 5 seconds	46	46.75
2 minutes 15 seconds	47	48
1 minute 30 seconds	54	55
1 minute 10 seconds	55	55.5
1 minute 10 seconds	55.5	56
1 minute 10 seconds	56	56.25
45 seconds	57	57.5

Bachmetjew (1) performed several experiments on the temperature of insects, especially *Lepidoptera*. He used a thermocouple made by soldering steel and manganese wires together. Both wires were connected to a galvanometer, one directly and the second after it had passed through a commutator. The wires were insulated by passing them through small glass tubes. Butterflies were placed on the couple, and from many experiments Bachmetjew concluded that:

When the body temperature reached  $39^{\circ}\text{C.}$  the insect became very active and died at  $46$  to  $47^{\circ}\text{C.}$

When the air temperature was lowered the body temperature of the insect was lowered to approximately  $-15^{\circ}\text{C.}$ ; there was then a rebound

in the body temperature from about  $-15^{\circ}$  to about  $-1^{\circ}$ . When the temperature of the body began to fall the insect died when the low point ( $-15^{\circ}$  C.) was reached the second time.

Bachmetjew (2) made a general study of insect temperature in which he covered the work previously done on this subject.

Brunnich (3) made a study of the temperature of the bee body and the bee brood. He used a thermocouple made by soldering a copper and platinum wire together, forming the warm junction of the couple. To the free end of the platinum wire, which was only a few centimeters in length, he soldered another copper wire. This second union acted as the cold junction of the couple. He used the room temperature for his cold junction and made no allowance for changes in the temperature of the room. He also used a telescope reading galvanometer which he stated was not sensitive enough, since 15 seconds were required before the maximum reading was reached. The bees were greatly weakened by piercing with this rough thermocouple and soon died. He found that the body temperature of adult workers went as high as  $39.6^{\circ}$  C., while that of the drones went as high as  $48.4^{\circ}$ . The results, however, gave no indication of uniformity, because some individuals gave high temperatures while others, under the same conditions, gave low temperatures.

Since platinum is a good conductor of heat, there is great danger in having the warm and cold junction separated by a piece of wire only a few centimeters in length. In piercing the bee the temperature of its body or the handling of the wires, if held near the second couple, is likely to increase the temperature of that couple, thereby introducing an error in the readings. The increasing of the temperature of the second couple was undoubtedly the cause of the wide range in the body temperature cited by Brunnich in his paper.

#### METHODS

The piercing of the bees in the following experiment was done with a thermocouple (Pl. I, A), made by soldering No. 20 double cotton covered copper and constantin (a copper-nickel alloy) wire together. The wires were tapered to a fine point before soldering by inserting the ends in concentrated nitric acid and slowly withdrawing them. This process was repeated until the wires obtained the desired points. The tapered ends were then soldered together and the surplus solder filed off. A piece of cork was inserted between the two wires near their junction to strengthen the couple and aid in the handling of it.

The readings were made with the aid of a pyrovolter (Pl. I, C) and galvanometer (Pl. I, D), and the bee was pierced with a thermocouple of which the cold junction (Pl. I, B) was placed in a thermos bottle filled with ice and water to keep it at  $0^{\circ}$  C. A Northrup pyrovolter which has scales graduated into millivolts and degrees centigrade and a Leeds-Northrup outside galvanometer to aid in the setting of the zero point were used. By having one junction of the thermocouple in ice and water the resulting reading on the pyrovolter was the actual temperature of the bee in degrees centigrade.

One of the rooms in a cold-storage plant was used for the low temperature of  $2.5^{\circ}$ ,  $5.5^{\circ}$ ,  $8^{\circ}$ , and  $9^{\circ}$  C. For "room temperature," readings were made in the laboratory. Temperature readings for  $27^{\circ}$ ,  $30.5^{\circ}$ ,  $35^{\circ}$ ,  $39.5^{\circ}$ ,  $43.5^{\circ}$ ,  $52^{\circ}$ , and  $58^{\circ}$  C. were made in a temperature box in

which the heat was regulated by a series of lights. The temperature box was made by placing insulite on the outside of the box 34 by 35 by 52 inches, the inside of which was lined with asbestos paper. There are two ventilating holes, one in the side near the bottom, the other in the top of the box.

At all temperatures the bees were allowed at least 10 minutes to become adapted to the surrounding air before the first of the group was pierced. When readings were made at 52° and 58° C. it was found that bees exposed to this high temperature for about 30 minutes were dead. The

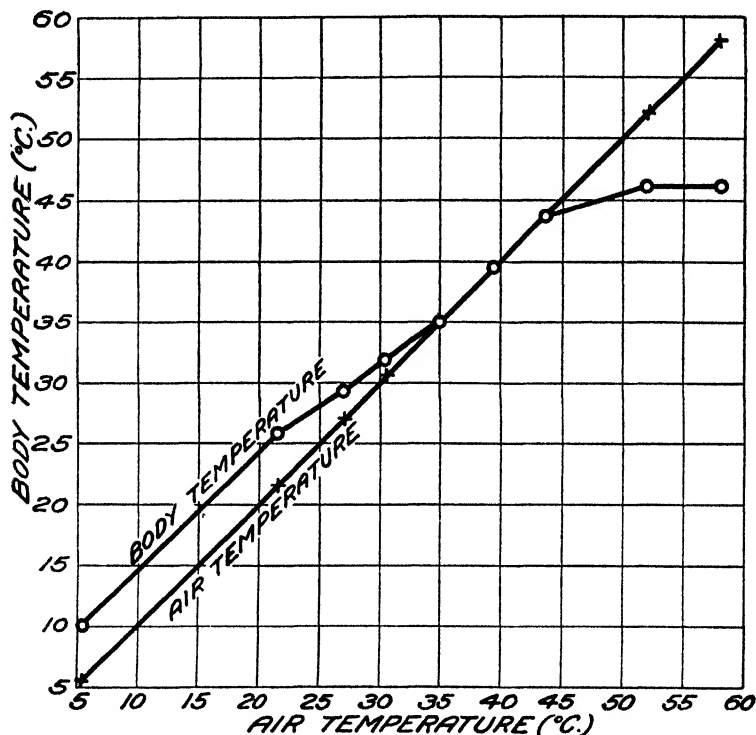


FIG. 1.—Graph showing the relation of body temperatures of Italian bees with the surrounding air. The bees which were taken from one hive were under winter conditions.

bees were placed in the temperature box at this high temperature in groups varying from 5 to 8 bees per group and exposed to the temperature for 10 minutes before any were pierced. The groups were placed in the box while its temperature was about that of the room. The lights were then turned on, and after the temperature had reached 52° or 58° and remained constant for 10 minutes the temperature readings were made.

The data for the temperature curve (fig. 1) were obtained from Italian bees that were all taken from one hive and in winter condition. On November 25 this hive along with about 90 others was placed in a

room in a cold-storage plant which maintained a temperature of about  $7^{\circ}$  to  $7.5^{\circ}$  C. throughout the experiment. A control was made by taking bees from different hives and placing them in groups, no two bees from the same hive in a group. A different group was used for each of the points on the curve.

Because of the popular belief that the Carniolian bees can withstand a lower temperature than the Italian bees it was thought advisable to make temperature readings on the Carniolians. Readings were taken at the temperatures of  $2.5^{\circ}$ ,  $5.5^{\circ}$ ,  $8^{\circ}$ ,  $9^{\circ}$ ,  $30^{\circ}$ ,  $35^{\circ}$ , and  $52^{\circ}$  C. A group of 10 Carniolians was placed in rooms having temperatures of  $2.5^{\circ}$ ,  $5.5^{\circ}$ , and  $9^{\circ}$  C. along with a group of 10 Italian bees. In piercing the bees an Italian was pierced first and then a Carniolian until all the bees were pierced. At  $8^{\circ}$ ,  $30^{\circ}$ ,  $35^{\circ}$ , and  $52^{\circ}$  the Carniolians were handled in the same manner as described for the Italians.

When taken from the hive the bees were placed in individual cages which allowed each bee the space of  $\frac{1}{4}$  by  $\frac{1}{4}$  by  $\frac{5}{8}$  inch. The cage was made from a block of wood  $\frac{3}{4}$  by  $\frac{3}{4}$  by 2 inches with a  $\frac{1}{4}$  by  $\frac{1}{4}$  inch groove in one surface. Cotton thread was used to wind around the block to prevent the escape of the bee. The thread also aided in piercing, as it permitted full view of the bee and eliminated the possibility of cross currents which might occur when using a wire screen to cover the groove. All piercing was done in the thorax, since there are no large air sacs in this region. It did not make any difference whether the dorsal or ventral surface was pierced, as is shown in Table I. By using a fine thermocouple there was no noticeable bad effect on the bee such as Brunnich (3) described in his paper. Several bees which were pierced, placed in a cage and fed, lived as long as bees not pierced and kept under the same conditions. Upon piercing these bees the second time, the same differences between the body temperature and that of the air were obtained as at the first piercing.

All thermocouples used in the experiment were carefully compared with the mercurial thermometer used by taking the readings of the temperature of the air before any of the bees were pierced. The following is an example of the results obtained in such comparison:

Air temperature (mercurial thermometer)  $24^{\circ}$  C.

Air temperature (thermocouple)  $23.5^{\circ}$ ,  $24^{\circ}$ ,  $24^{\circ}$ ,  $24^{\circ}$ ,  $24^{\circ}$ ,  $24^{\circ}$ ,  $23.5^{\circ}$ ,  $24^{\circ}$ ,  $24^{\circ}$ ,  $24^{\circ}$  C.

TABLE 1.—Body temperatures of bees at air temperature 5.5° C.

Bee No.	Body temperature.	Surface pierced.	Condition of bee.	Bee No.	Body temperature.	Surface pierced.	Condition of bee.
	°C.				°C.		
1.	11	Ventral...	Active	51	10	Ventral	Inactive.
2	11	Dorsal...	Do	52	9	do	Do
3	12	do	Do	53	9	Dorsal	Do.
4	11	do	Do	54	9	do	Do.
5	10.5	do	Do	55	9.5	do	Do.
6	11	do	Inactive	56	10	do	Do.
7	10	Ventral	Do.	57	10.5	do	Do
8	12	Dorsal	Do	58	10	do	Do.
9	11	Ventral	Do	59	10.5	do	Do.
10	11	do	Do	60	10	do	Do.
11	11.5	Dorsal	Do.	61	10.5	do	Active.
12	14	do	Do	62	13.5	do	Do.
13	12	Ventral	Do	63	10.5	do	Do
14	11	Dorsal	Do.	64	10	Ventral	Do.
15	11	Ventral	Do	65	9	Dorsal	Do.
16	10.5	do	Active.	66	9.5	Ventral	Do.
17	11	Dorsal	Do	67	9.5	do	Inactive.
18	11	do	Do	68	10	do	Do.
19	10.5	do	Do	69	9	Dorsal	Do
20	10.5	Ventral	Do	70	9.5	Ventral	Active.
21	10	Dorsal	Do	71	13.5	do	Do
22	9	do	Inactive	72	11	do	Inactive.
23	9.5	do	Do	73	10	do	Do
24	9.5	Ventral	Do.	74	10	Dorsal	Do
25	10	Dorsal	Do	75	9	do	Do
26	10	Ventral	Do	76	10	do	Active.
27	10	Dorsal	Do	77	10	do	Do
28	10	Ventral	Do	78	9	do	Do.
29	11	Dorsal	Do	79	10	do	Do.
30	9.5	do	Do	80	10	do	Do
31	8.5	do	Active	81	10	Ventral	Do
32	9	Ventral	Do	82	9.5	do	Inactive.
33	9	do	Do	83	10.5	do	Do
34	9.5	do	Do	84	10.5	do	Do
35	9.5	do	Do	85	10	do	Do.
36	9	Dorsal	Do	86	11.5	Dorsal	Do.
37	9	do	Do	87	11	do	Do
38	9	do	Inactive	88	11	do	Do
39	9.5	Ventral	Do	89	10.5	Ventral	Do
40	9	Dorsal	Do	90	10.5	Dorsal	Do
41	10	Ventral	Do	91	9.5	do	Active
42	9	do	Do	92	10	Ventral	Do
43	9.5	Dorsal	Do	93	9	Dorsal	Do
44	9.5	do	Do	94	9.5	do	Do.
45	9.5	Ventral	Do	95	9	do	Do.
46	10	do	Active	96	9.5	Ventral	Do
47	10.5	do	Do	97	9.5	Dorsal	Inactive.
48	9.5	do	Do	98	9	do	Do.
49	9	do	Do	99	9.5	do	Do.
50	10.5	do	Do	100	9.5	Ventral	Do.

Average body temperature 10.2° C. Difference between average body temperature and air temperature 4.7° C.

#### TEMPERATURE OF THE ITALIAN WORKERS

A striking result noted in the tables was the small range in the differences between the maximum and minimum temperature readings at the different temperatures, as is shown in Table II. This is especially noticeable at the higher temperatures. In Table I there is a range of 5.5° C. between the maximum and minimum temperatures in the readings of 100 bees. We find in Table II a variation in the differences from 9° at 21.4° to 1.5° at 27° and 43.5° with the difference of 2.5° at the temperatures of 30.5°, 35°, 52°, and 58°.

TABLE II.—*Body temperatures of bees at various air temperatures*

Air temperature.	Average body temperature.	Difference between body and air temperature.	Maximum body temperature.	Minimum body temperature.	Number of bees pierced.
5.5	10.2	4.7	14	8.5	100
21.4	25.8	4.4	31	22.0	100
27.0	29.1	2.1	30	28.5	54
30.5	32.0	1.5	34	31.5	100
35.0	35.1	.1	37	34.5	100
39.5	39.5	.0	42	38.0	100
43.5	43.6	.1	44	42.5	100
52.0	46.0	-6.0	48	45.5	100
58.0	46.4	-11.6	48	45.5	11

That bees are capable of regulating their temperature for a short period of time was shown by the fact that when bees were placed in the temperature box at 52° and 58° C. their body temperature did not at once correspond to that of the air, as it did from 35° to 44°, but was lower. After the bees were exposed to this temperature for about .25 minutes, their body temperature approached nearer that of the air temperature. After they were in the temperature box for 30 minutes, they were dead and had the same temperature as that of the air. A few bees were tried at a low temperature - 10° to +21°, and it was noted that the temperature of the body fell with that of the environment.

By handling the bees in individual cages they were not excited before piercing. The space allotted to each bee was large enough to permit it some movement, but not large enough for it to make use of its wings. Throughout the entire experiment only two bees, No. 62 and 71, in Table I, were observed to be fanning. They were immediately pierced and found to give a reading of 8° C. above that of the room, while those not fanning had a temperature about 4.6° above the air temperature. The bees were pierced at a low temperature and in the individual cages, so that the heat produced by the fanning was conserved, thereby raising their own temperature.

When bees were exposed to the temperature of 5.5° C., they were taken into the room in groups of 15. A period of three minutes elapsed before the piercing of each successive bee to permit the thermocouple to return to room temperature. After the piercing of about the sixth bee the remainder of them became motionless but were easily revived by holding them in the hand for a few minutes. After a group of 10 bees was kept in the room for a period of two hours they were pierced, and they gave an average temperature of 2° above the room temperature. After the bees were kept at this low temperature for 48 hours they were dead and recorded the same temperature as the surrounding air. The bees may have died from either starvation or from cold.

When readings were taken at "room temperature" the work was done in the laboratory and the temperature varied from 19.5° to 24° C., with an average of 21.5°. The bees were brought into the laboratory in groups of 10. At all other temperatures, with the exceptions of 52° and 58°, the bees were used in groups of 25.

As the air temperature was increased, the difference between the body temperature of the bee and the temperature of the air decreased until at

35°, or brood-rearing temperature, the two corresponded. As the air temperature went up from 35° to 44° the temperature of the bee and that of the surrounding air were the same, as shown in Table II.

When readings were taken at 52° and 58° C. the time factor entered, as the bees were at the point of their fatal temperature (about 46° to 48°). A bee was placed in the temperature box for 20 minutes before piercing. The thermocouple was placed in the thorax and kept there for seven minutes, readings being recorded every minute, during which time all movement ceased. Seven minutes later, when the couple was again inserted in the thorax, the body temperature of the bee was identical with that of the surrounding air. For the 100 bees used at 52° there was an average body temperature of 46°. If left at this temperature for 30 minutes the bees died, which indicated that the fatal temperature was around 46° to 48°.

About 250 bees were placed in a half pint (250-cc.) bottle. A thermocouple inserted near the top of the bottle gave a reading of 32° C. The temperature of the air in the room was at 25°. When the thermocouple was inserted farther into the bottle, so that the bees clustered on the wires, the thermocouple registered 34°. Later, when the bees were quiet, the couple was reinserted and the temperature was at 29°. The bees were agitated and the temperature went up to 32°. Temperature readings were taken of individual bees, while the cluster was at 32°, and they gave an average temperature reading of 34°.

Ten of these bees placed in a 10-cc. vial gave a temperature reading of 32° C. When the bees were quiet, they had a temperature of 29°. Agitating the bees caused their temperature to rise slowly until it reached 32° and remained there as long as the bees were active. This demonstrated that the bees can effect the temperature of their environment. If the heat radiated by the bee can be conserved, the temperature of the environment will go up. This in turn will permit the bee to raise its own temperature correspondingly, as there is a constant relationship between the body temperature of the bee and that of the environment it is in.

As is shown by the temperature curve (fig. 1) the supply of oxygen was a large factor in the amount of radiation that was set up by the bees. If the oxygen is limited, as may be done by corking the bees up tight in a bottle, the bees become inactive and the temperature goes down. If, on the other hand, the cork is removed and fresh oxygen is admitted, the bees give an immediate response.

#### TEMPERATURE OF ITALIAN DRONES

A few temperature readings were made on the drones which were taken from the hive early in the morning before they had left the hive to fly. At room temperature, 23° C., the body temperature of the drones corresponded to that of the workers, while at 52° the drones, average temperature was 42.4° compared with 46° for the workers.

#### TEMPERATURE OF CARNIOLIAN WORKERS

In work on the Carniolians more emphasis was placed on the lower temperature than on the higher ones. In Table III we find that there is no difference between the Carniolian and Italian bees for the temperatures of 2.5°, 5.5°, and 9° C. A comparison of Tables II and III for the temperatures of 30°, 35°, and 52° gives the same results.

TABLE III.—*Comparison of body temperatures of Carniolian and Italian bees at various air temperatures*

Number of bees pierced.		Air temperature.	Average body temperature.		Difference between body and air temperatures.		Maximum body temperature.		Minimum body temperature.	
Carniolians.	Italians.		Carniolians.	Italians.	Carniolians.	Italians.	Carniolians.	Italians.	Carniolians.	Italians.
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
10	10	2.5	4.9	5.1	2.4	2.6	7	6	4	4.5
8	8	5.5	8.5	8.5	3.0	3.0	9	9	8	8.0
.....	.....	8.0	13.2	.....	5.2	.....	16	.....	11.5	25.0
10	10	9.0	13.8	13.6	4.8	4.6	16.5	16.0	12.5	12.5
25	.....	30.0	32.1	.....	2.1	.....	33.0	.....	31.5	.....
25	.....	35.0	35.0	.....	0.0	.....	35.0	.....	34.5	.....
25	.....	52.0	46.3	.....	-5.7	.....	48.0	.....	45.5	.....

## FREEZING POINT

A study of the freezing point and the phenomenon of supercooling of the bee was made. An ether bath and a potentiometer were used. The ether bath was more easily controlled than an ice bath, and the temperature dropped about 0.5° C. per minute. Care was taken not to move the bee or have it come in contact with anything, as the least amount of movement will bring about the freezing of the insect. If the insect is moved or shaken about the time the rebound from supercooling to the actual freezing point is to take place, the bee will freeze and the rebound is not evident. It was also found that if the temperature of the bee when nearing its freezing point went down slowly, the freezing would take place and there would be no supercooling with a rebound to the freezing point. However, if the temperature went down rapidly as it neared the freezing point, the bee was supercooled and the rebound occurred. Three individual bees were taken down to -2.3°, when there was a rebound to -0.8°, or the actual freezing point. Another bee was supercooled to -4.3°, when the rebound occurred, the temperature going up to -2°, the actual freezing point. This conforms with the results obtained by Bachmetjew. The variation in the results is probably due to the fact that individuals do not have the same freezing point.

## SUMMARY

The large variation in the results obtained by the early workers was due to the differences in methods of performing their experiments. Many of the old workers used mercurial thermometers, or thermocouples, which were then just coming into use. Nobili and Melloni (15) were the first to ever use this method. Because of the wide variation and unrefinement of the methods used by the early workers and the results they obtained their work remains only of historical value.

Bachmetjew (2) has contributed considerably to the general work on insect temperature by bringing together and giving a summary of the older works. In addition to this, he has performed many original experiments on the temperature of individual insects.

Brunnich (3) is the most recent worker on the temperature of bees. The lack of uniformity in his results is undoubtedly due to the fact that



he used the room temperature for the cold junction of his thermocouple. This cold junction was not controlled at a constant temperature, but was subject to fluctuations due to changes in the temperature of the room and also to the heat conducted along the short platinum wire from the bee which was pierced and from the hand with which the thermocouple was held while piercing the bees and taking the readings. Thus fluctuations in readings which Brunnich attributed to fluctuations of the temperature of the bee were more probably due to the fluctuations in the temperature of the cold junctions.

The author obtained very uniform results by using refined thermocouple methods and by having the cold junction in ice and water, keeping it constantly at 0° C.

### CONCLUSIONS

From the foregoing results the following conclusions were made:

The average body temperature of the bee is 4.7° C. above that of the surrounding air when the air temperature is 5.5° and coincides with the air temperature when that is between 35° and 44°.

At 52° C. or above the temperature of the bee's body is lower than that of the air if not exposed to the high temperature for a long period of time.

The maximum fatal temperature of bees is about 46° to 48° C., and the freezing point is about -1°.

There is no appreciable difference between the body temperature of the Carniolian and Italian bees.

Bees are not wholly subject to the temperature of their environment, but are capable within certain limits of regulating their body temperature.

The ability of a colony to regulate its temperature is undoubtedly due to the ability of the individual to regulate its body temperature plus the ability to regulate and conserve the heat produced.

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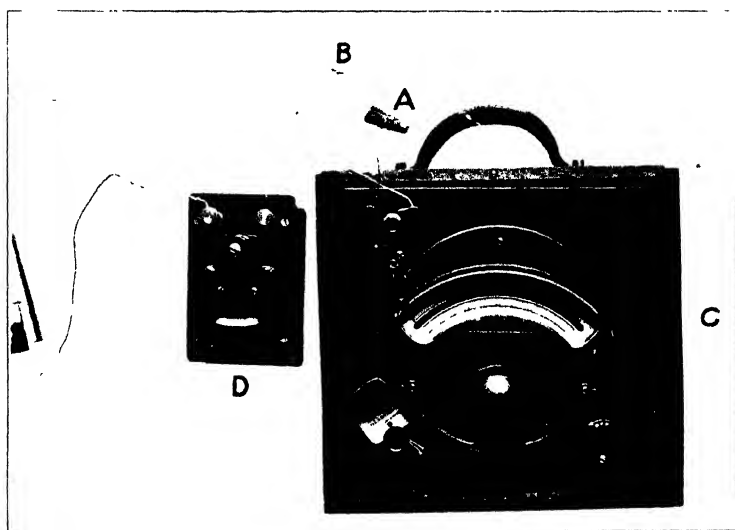
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PLATE I

- A.—Warm junction of the thermocouple.
- B.—Cold junction of the thermocouple.
- C.—Pyrovolter.
- D.—Galvanometer.

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# A STUDY OF THE EFFECT OF CHANGING THE ABSOLUTE REACTION OF SOILS UPON THEIR AZOTOBACTER CONTENT <sup>1</sup>

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## INTRODUCTION

In studying the apparent correlation between the absolute reaction of soils and the presence of *Azotobacter* it occurred to the writer that if such a correlation existed one ought to be able, by varying the reaction, to control the *Azotobacter* content of any soil. To test the correctness of this view three lines of investigation were suggested.

(1) If two soils, one with a hydrogen-ion concentration favorable to the growth of *Azotobacter*, the other unfavorable, are mixed in varying proportions the hydrogen-ion concentration of some mixtures should be favorable and others unfavorable to the growth or existence of this group of organisms. All mixtures giving a hydrogen-ion concentration less than the maximum tolerated by *Azotobacter* should, upon subsequent analyses, show their presence. On the other hand, all mixtures giving a hydrogen-ion concentration greater than the maximum should not show the presence of *Azotobacter*, provided a sufficient incubation period elapsed to bring about their destruction.

(2) If the absence of *Azotobacter* in any soil is due to its high hydrogen-ion concentration, one should be able to decrease the acidity by the addition of increasing quantities of nontoxic basic materials, to a point where, once introduced, *Azotobacter* would survive.

(3) If a high hydrogen-ion concentration is inimical to the presence of *Azotobacter* one should be able, by the addition of acid, to increase the acidity of any soil containing *Azotobacter* to a point beyond the maximum tolerated by these organisms and thereby cause their disappearance.

The purpose of this paper is to report a few typical examples of a large number of experiments that have been carried out along these lines.

## METHODS

The methods employed in the experiments here reported were similar to those previously reported.<sup>2</sup> Tests for *Azotobacter* were made by inoculating a mannite cultural solution with 10 per cent of soil, or the equivalent, as a suspension. Incubation was at room temperature. At frequent intervals during incubation the cultures were examined macroscopically to ascertain whether or not a film characteristic of *Azotobacter* was present. If the film was not typical and there were any indications of *Azotobacter* being present a microscopic examination was made. If the evidence thus obtained showed the presence of *Azotobacter*, it has been indicated by a plus sign in the following tables. A minus sign

<sup>1</sup> Accepted for publication June 29, 1922. Contribution No. 48, Department of Bacteriology, Kansas Agricultural Experiment Station.

<sup>2</sup> GAINNEY, P. L. SOIL REACTION AND THE GROWTH OF AZOTOBACTER. *In Jour. Agr. Research*, v. 14, p. 265-271. 1918.

signifies the absence of *Azotobacter*. Where a question mark has been inserted it was impossible to tell whether *Azotobacter* were present.

The hydrogen-ion concentration determinations were made either colorimetrically after Gillespies method,<sup>3</sup> or electrometrically upon suspension of the soil. For the latter purpose a Leeds and Northrup potentiometer outfit was used in connection with saturated K Cl—calomel and hydrogen electrodes similar to that described by Hildebrand.<sup>4</sup> In general the two methods agreed very closely.

The mixtures of soils, or soils with various additions, were placed in 500-cc. wide-mouthed bottles plugged with cotton, and the moisture content was brought to a favorable point. Incubation was at room temperature. At frequent intervals the moisture lost through evaporation was restored. A more detailed description of the methods employed will be presented when the data are published in full.

## RESULTS

### MIXING SOILS

In the two experiments reported in Tables I and II, two soils (A and E), containing a vigorous *Azotobacter* flora, and one soil (B), in which *Azotobacter* have never been found, were studied. Soil A is approximately neutral,  $P_H$  6.94. Soil E contains a high percentage of limestone and is alkaline,  $P_H$  7.73. Soil B is strongly acid,  $P_H$  3.65. Soils A and B would, under normal conditions, be expected to have approximately the same reaction, since the two are from similar locations and were taken only a few yards apart. However, the soil where B was taken was planted to pine trees a number of years ago and the high acidity is undoubtedly due to the decomposition of the highly acid pine needles. Extensive use has been made of this soil because it is the only strongly acid soil yet located in this immediate vicinity.

Previously reported experiments,<sup>5</sup> indicated that the maximum hydrogen-ion concentration tolerated by *Azotobacter* in soils is near  $1 \times 10^{-6}$ , or  $P_H$  6.0. If  $P_H$  6.0 represents the maximum acidity tolerated by this group of organisms, then all mixtures of soils A and B or E and B less acid than  $P_H$  6.0 should, upon subsequent analyses, show *Azotobacter*, while all mixtures in which the acidity was very much greater than  $P_H$  6.0 should not show their presence. It is probable that *Azotobacter* can exist for some time in a hydrogen-ion concentration that would not permit growth. This being true, some of the less acid samples in which *Azotobacter* can not grow might be able to initiate the growth of *Azotobacter* in a cultural solution. It would be expected that the higher the hydrogen-ion concentration the more rapidly the *Azotobacter* would be destroyed. From the relative reactions of the three soils it would also be expected that the quantity of soil A necessary to add to soil B in order to reduce the acidity of the mixture to  $P_H$  6.0 would be larger than would be required in soil E. A glance at the second, third, and fourth columns of Tables I and II will show that in mixtures of soils A and B the ratio of A to B required to give an acidity less than  $P_H$  6.0 lies somewhat between 9 to 1 and 4 to 1. In mixtures of soils E and B the ratio of E to B required to give the same reaction is 1 to 3.

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<sup>4</sup> HILDEBRAND, J. H. SOME APPLICATIONS OF THE HYDROGEN ELECTRODE IN ANALYSIS, RESEARCH, AND TEACHING. *J. Jour. Amer. Chem. Soc.*, v. 35, p. 847-871, 15 fig. 1913

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TABLE I.—*Effect of mixing an alkaline and an acid soil upon presence of Azotobacter. Mixtures of soils A and B*<sup>a</sup>

Sample No.	Grams of soil A.	Grams of soil B.	pH.	Azotobacter cultures					
				No CaCO <sub>3</sub> added to soil			CaCO <sub>3</sub> added to soil		
				Jan 30.	May 22.	Dec 11.	Jan 30.	May 22.	Dec 11.
1.....	300	0	7.02	+	+	+	+	+	+
2.....	300	0	6.86	+	+	+	+	+	+
3.....	270	30	6.42	+	+	+	+	+	+
4.....	270	30	6.32	+	+	+	+	+	+
5.....	240	60	5.76	+	+	+	+	+	+
6.....	240	60	5.82	+	+	+	+	+	+
7.....	150	150	4.60	+	+	—	+	+	+
8.....	150	150	4.61	+	—	—	+	+	+
9.....	60	240	4.11	+	—	—	+	+	+
10.....	60	240	4.09	+	—	—	+	+	+
11.....	30	270	3.79	+	—	—	+	+	+
12.....	30	270	3.90	+	—	—	+	+	+
13.....	15	285	3.77	+	—	—	+	+	+
14.....	15	285	3.75	+	—	—	+	+	+
15.....	3	297	3.75	+	—	—	+	+	+
16.....	3	297	3.68	+	—	—	+	+	+
17.....	1	299	3.66	+	—	—	+	+	+
18.....	1	299	3.65	+	—	—	+	+	+
19.....	0	300	3.68	—	—	—	—	—	—
20.....	0	300	3.62	—	—	—	—	—	—

<sup>a</sup> + = Presence of Azotobacter

— = Absence of Azotobacter

Experiment set up Jan 30. Moisture content optimum. Acidity determined electrometrically.

TABLE II.—*Effect of mixing an alkaline and an acid soil upon presence of Azotobacter. Mixtures of soils E and B*<sup>a</sup>

Sample No	Grams of soil E	Grams of soil B	pH	Azotobacter cultures		
				May 1	May 18.	June 21.
1.....	200	0	7.73	+	+	+
2.....	199	1	7.64	+	+	+
3.....	195	5	7.67	+	+	+
4.....	190	10	7.67	+	+	+
5.....	175	25	7.56	+	+	+
6.....	150	50	7.61	+	+	+
7.....	125	75	7.52	+	+	+
8.....	100	100	7.37	+	+	+
9.....	75	125	7.06	+	+	+
10.....	50	150	6.02	+	+	+
11.....	25	175	4.87	+	+	—
12.....	10	190	3.99	+	?	—
13.....	5	195	3.94	+	+	—
14.....	1	199	3.74	—	—	—
15.....	0	200	3.64	—	—	—

<sup>a</sup> ? = Azotobacter indefinite.

+ = Presence of Azotobacter

— = Absence of Azotobacter.

Experiment set up May 1. Moisture content optimum. Acidity determined electrometrically.



A further examination of the two tables will show that, when cultured immediately after mixing, all mixtures of A and B and E and B, except 1 part of E to 199 parts of B, contained *Azotobacter*. Subsequent cultures, made after varying periods of incubation, show a gradual disappearance of *Azotobacter* in the more acid mixtures until only those samples contain them in which the acidity approaches very close to or is less than  $P_H$  6.0. Numerous experiments have shown that the more acid a soil is the quicker will *Azotobacter* disappear therefrom when introduced, and that they can exist for some time in soils only slightly more acid than  $P_H$  6.0. All the evidence accumulated so far, however, indicates that they can not remain indefinitely in a soil very much more acid than  $P_H$  6.0.

#### ADDITION OF CALCIUM CARBONATE

In these experiments the aim was to find out whether lowering the hydrogen-ion concentration of a soil more acid than  $P_H$  6.0, and not containing *Azotobacter*, would render the soil capable of supporting such a flora when introduced. The method followed was to add increasing quantities of various basic materials, particularly calcium carbonate, to acid soils containing no *Azotobacter*, inoculate the soil with *Azotobacter*, and after varying incubation periods culture to see whether or not the introduced organisms survived.

TABLE III.—*Effect of  $CaCO_3$  upon the longevity of *Azotobacter* in an acid soil (B)*<sup>a</sup>

Sample No	Percentage of $CaCO_3$	Pounds $CaCO_3$ per acre <sup>b</sup>	Azotobacter cultures			
			Sept. 1	Oct. 21	Jan. 4	Feb. 18.
1.....	0.0	0	+	+	—	—
2.....	.1	3,000	+	+	—	—
3.....	.2	6,000	+	+	—	—
4.....	.3	9,000	+	+	—	—
5.....	.4	12,000	+	+	—	—
6.....	.5	15,000	+	+	—	—
7.....	.6	18,000	+	+	+	+
8.....	.7	21,000	+	+	+	+
9.....	.8	24,000	+	+	+	+
10.....	.9	27,000	+	+	+	+
11.....	1.0	30,000	+	+	+	+
12.....	1.1	33,000	+	+	+	+
13.....	1.2	36,000	+	+	+	+
14.....	1.3	39,000	+	+	+	+
15.....	1.4	42,000	+	+	+	+
16.....	1.5	45,000	+	+	+	+

<sup>a</sup> + = Presence of *Azotobacter*. — = Absence of *Azotobacter* Experiment set up Aug 20 Moisture content optimum

<sup>b</sup> 3,000,000 pounds soil.

In Table III it will be observed that a minimum of 18,000 pounds per acre (3,000,000 pounds), or 0.6 per cent calcium carbonate, were required to render soil B capable of supporting an *Azotobacter* flora. Unfortunately the hydrogen-ion concentration of the samples of soil of these experiments was not determined.

TABLE IV.—*Effect of basic compounds upon longevity of Azotobacter in an acid soil (B)<sup>a</sup>*

Sample No.	Basic compounds added	Pounds per acre <sup>b</sup>	P <sub>H</sub> .	Azotobacter cultures.		
				Feb 9	Mar 18	May 7.
1	0	.....	4.3	—	—	—
2	0	.....	4.3	—	—	—
3	0.01 per cent CaCO <sub>3</sub>	300	4.3	—	—	—
4	.05 per cent CaCO <sub>3</sub>	1,500	4.4	—	—	—
5	.10 per cent CaCO <sub>3</sub>	3,000	4.7	—	—	—
6	.50 per cent CaCO <sub>3</sub>	15,000	5.3	+	—	—
7	1.00 per cent CaCO <sub>3</sub>	30,000	6.5	+	+	+
8	2.50 per cent CaCO <sub>3</sub>	75,000	7.0	+	+	+
9	1.01 per cent Na <sub>2</sub> CO <sub>3</sub>	300	4.3	—	—	—
10	.05 per cent Na <sub>2</sub> CO <sub>3</sub>	1,500	4.8	—	—	—
11	.10 per cent Na <sub>2</sub> CO <sub>3</sub>	3,000	5.2	—	—	—
12	.50 per cent Na <sub>2</sub> CO <sub>3</sub>	15,000	5.8	—	—	—
13	1.00 per cent Na <sub>2</sub> CO <sub>3</sub>	30,000	6.5	—	—	—
14	2.50 per cent Na <sub>2</sub> CO <sub>3</sub>	75,000	8.6	—	—	—
15	.01 per cent Mg CO <sub>3</sub>	300	4.3	—	—	—
16	.05 per cent Mg CO <sub>3</sub>	1,500	4.3	—	—	—
17	.10 per cent Mg CO <sub>3</sub>	3,000	4.3	—	—	—
18	.50 per cent Mg CO <sub>3</sub>	15,000	5.4	+	—	—
19	1.00 per cent Mg CO <sub>3</sub>	30,000	6.6	+	+	+
20	2.50 per cent Mg CO <sub>3</sub>	75,000	7.8	+	—	—

<sup>a</sup> + = Presence of Azotobacter — = Absence of Azotobacter. Experiment set up Jan 21, Moisture content optimum

<sup>b</sup> 3,000,000 pounds soil

<sup>c</sup> Greater than 8.6

The data reported in Table IV were secured from the same soil, but the variations in the quantity of basic material added were much greater. Here again 15,000 pounds of calcium, magnesium, or sodium carbonate failed to reduce the acidity to P<sub>H</sub> 6.0 or to make conditions favorable for the existence of Azotobacter. The P<sub>H</sub> where 15,000 pounds of calcium carbonate were added was 5.3. Where 30,000 pounds, the next largest quantity, were added the P<sub>H</sub> was 6.5 and the sample contained Azotobacter at all subsequent analyses.

Soil G with a reaction very close to P<sub>H</sub> 5.8 (colorimetrically) was used in the experiments reported in Table V. The quantity of calcium, sodium, or magnesium carbonate necessary to reduce the acidity to P<sub>H</sub> 6.0, or less, was not as great as was required by soil B. Two months after incubation started all samples except No. 12 (Table V) contained Azotobacter. In this particular case the high concentration of magnesium carbonate had apparently destroyed all organisms of this group. At the next analyses, seven weeks later, Azotobacter had disappeared from all samples more acid than P<sub>H</sub> 6.0 and still remained in all those with a less high hydrogen-ion concentration, except when apparently destroyed by the high concentration of magnesium and sodium carbonate.

When excessive quantities of sodium and magnesium carbonate were added they apparently became toxic to the Azotobacter. The quantity necessary to produce such a condition was greater in soil G than in soil B. Lipman<sup>6</sup> has reported a toxic effect upon Azotobacter from both sodium and magnesium carbonate.

<sup>6</sup> LIPMAN, Charles B., and SHARP, L. T. TOXIC EFFECTS OF "ALKALI SALTS" IN SOILS ON SOIL BACTERIA. III. NITROGEN FIXATION. *1/2 Centbl. Bakt. [etc.]*, Abt. 2, Bd 35, p 647-655, 1 fig 1912

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TABLE V.—*Effect of basic compounds upon the longevity of Azotobacter in an acid soil (J)*<sup>a</sup>

Sample No	Basic compounds added.	Pounds per acre <sup>b</sup>	P <sub>H</sub> .	Azotobacter cultures.		
				Feb. 10.	Mar. 19.	May 8.
1	0.01 per cent CaCO <sub>3</sub> .....	300	5.9	+	+	—
2	.05 per cent CaCO <sub>3</sub> .....	1,500	6.0	+	+	—
3	.10 per cent CaCO <sub>3</sub> .....	3,000	6.2	+	+	+
4	.50 per cent CaCO <sub>3</sub> .....	15,000	7.4	+	+	+
5	1.00 per cent CaCO <sub>3</sub> .....	30,000	7.4	+	+	+
6	2.50 per cent CaCO <sub>3</sub> .....	75,000	7.4	+	+	+
7	.01 per cent Na <sub>2</sub> CO <sub>3</sub> .....	300	5.8	+	+	—
8	.05 per cent Na <sub>2</sub> CO <sub>3</sub> .....	1,500	6.0	+	+	+
9	.10 per cent Na <sub>2</sub> CO <sub>3</sub> .....	3,000	6.2	+	+	+
10	.50 per cent Na <sub>2</sub> CO <sub>3</sub> .....	15,000	7.6	+	+	+
11	1.00 per cent Na <sub>2</sub> CO <sub>3</sub> .....	30,000	c 8.6	+	+	+
12	2.50 per cent Na <sub>2</sub> CO <sub>3</sub> .....	75,000	c 8.6	+	—	—
13	.01 per cent Mg.CO <sub>3</sub> .....	300	5.8	+	+	—
14	.05 per cent Mg.CO <sub>3</sub> .....	1,500	6.0	+	+	+
15	.10 per cent Mg.CO <sub>3</sub> .....	3,000	6.4	+	+	+
16	.50 per cent Mg.CO <sub>3</sub> .....	15,000	7.6	+	+	+
17	1.00 per cent Mg.CO <sub>3</sub> .....	30,000	8.0	+	+	+
18	2.50 per cent Mg.CO <sub>3</sub> .....	75,000	8.6	+	+	—

<sup>a</sup> + = Presence of Azotobacter. — = Absence of Azotobacter Experiment set up Jan 21. Moisture content optimum Acidity determined colorimetrically

<sup>b</sup> 3,000,000 pounds soil

<sup>c</sup> Greater than 8.6.

It is evident from the data presented in Tables III, IV, and V that the addition of calcium carbonate to an acid soil, not containing Azotobacter, if in sufficient quantity to reduce the hydrogen-ion concentration to approximately P<sub>H</sub> 6.0 or lower, is all that is necessary to render the soil capable of supporting an Azotobacter flora. In no case, however, have Azotobacter been observed to appear in a soil so treated unless accompanied by inoculation either natural or artificial. In other words, Azotobacter are actually not present and must be introduced or the addition of calcium carbonate can have no effect upon nitrogen fixation by this group of organisms.

#### EFFECT OF ADDING ACID

In the experiments reported in Tables VI and VII, increasing quantities of various acids were added to soils A and C. Both these soils contained abundant Azotobacter. The reaction of soil A was very near P<sub>H</sub> 7.0 and of soil C was near P<sub>H</sub> 6.5.

Sulphuric, hydrochloric, acetic, and butyric acids were added to soil C reported in Table VI. Sulphuric and hydrochloric acid increased the hydrogen-ion concentration of the soil as the quantity of acid increased. As soon as the acidity passed much beyond P<sub>H</sub> 6.0 Azotobacter were unable to survive prolonged incubation. When acetic or butyric acid was added it apparently had no effect upon the reaction, unless to reduce the hydrogen-ion concentration. This is contrary to the data presented in Table VII and is probably due to the time at which the hydrogen-ion concentration determinations were made. In Table VI the reaction was determined at the end of the period of incubation, while in Table VII the acidity determinations were made immediately

after being added or at the beginning of incubation. There are in soils organisms capable of utilizing many organic acids or their salts as food. This would cause the disappearance of the acid.

TABLE VI.—*Effect of adding acid to a soil containing Azotobacter (soil C)*<sup>a</sup>

Sample No.	Acid added to 200 gm soil	pH	Azotobacter cultures.	
			Mar 2	June 23
1.....	0.2 cc. <i>N/1</i> sulphuric.....	6.53	+	+
2.....	1.0 cc. <i>N/1</i> sulphuric.....	6.73	+	+
3.....	5.0 cc. <i>N/1</i> sulphuric.....	5.88	+	+
4.....	10.0 cc. <i>N/1</i> sulphuric.....	5.29	+	(?)
5.....	20.0 cc. <i>N/1</i> sulphuric.....	4.74	+	—
6.....	.2 cc. <i>N/1</i> acetic.....	6.42	+	+
7.....	1.0 cc. <i>N/1</i> acetic.....	6.61	+	+
8.....	5.0 cc. <i>N/1</i> acetic.....	6.64	+	+
9.....	10.0 cc. <i>N/1</i> acetic.....	6.74	+	(?)
10.....	20.0 cc. <i>N/1</i> acetic.....	7.10	—	(?)
11.....	.2 cc. <i>N/1</i> butyric.....	6.44	+	+
12.....	1.0 cc. <i>N/1</i> butyric.....	6.58	+	+
13.....	5.0 cc. <i>N/1</i> butyric.....	6.69	+	+
14.....	10.0 cc. <i>N/1</i> butyric.....	6.70	+	+
15.....	20.0 cc. <i>N/1</i> butyric.....	7.05	—	+
16.....	.2 cc. <i>N/1</i> hydrochloric.....	6.41	+	+
17.....	1.0 cc. <i>N/1</i> hydrochloric.....	6.51	+	+
18.....	5.0 cc. <i>N/1</i> hydrochloric.....	5.58	+	—
19.....	10.0 cc. <i>N/1</i> hydrochloric.....	5.51	+	—
20.....	20.0 cc. <i>N/1</i> hydrochloric.....	4.51	+	—
21.....	0.....	6.50	+	+

<sup>a</sup> + = Presence of Azotobacter

— = Absence of Azotobacter

? = Azotobacter indefinite

Experiment set up Feb. 16 Moisture content optimum.

Acidity determined electrometrically

The increased growth of such organisms would bring about the accumulation of protein in their bodies which, upon decomposition, would result in the formation of more ammonia and hence a possible decrease in the hydrogen-ion concentration. The initial high acidity might result in either the partial or total destruction of the Azotobacter flora. If only a few organisms survived they might escape detection by the methods used. As soon, however, as the reaction again became favorable they would become abundant and be easily detected. This is probably what occurred in samples 14 and 15 of Table VI. On the other hand, if all the Azotobacter were killed they would never reappear unless inoculation took place, and subsequent analyses would fail to reveal their presence even though the reaction were favorable. The rapidity with which the Azotobacter are destroyed probably depends upon the degree of acidity, while the completeness with which they disappear from an acid soil depends upon the length of time the organisms are in contact with the acid condition. In the examples just given their complete destruction would depend upon the initial acidity and the period that elapsed before the hydrogen-ion concentration was again reduced below the maximum tolerated.

The data in Table VII show that with increasing quantities of organic as well as mineral acids the hydrogen-ion concentration increases. From

the reaction of samples 6 and 18 in Table VII Azotobacter would not be expected to be present. For reasons just discussed, it is probable that their presence in these samples is due to the fact that the initial hydrogen-ion concentration did not exist sufficiently long to destroy them.

TABLE VII.—Effect of adding acid to soil containing Azotobacter (soil A) <sup>a</sup>

Sample No.	Acid added to 200 gm soil	P <sub>H</sub> .	Azotobacter cultures	
			Aug 22.	Oct 10.
1.....	0 .....	6.81	+	+
2.....	10 cc. 2N sulphuric .....	2.52	—	—
3.....	20 cc. 2N sulphuric .....	2.26	—	—
4.....	50 cc. 2N sulphuric .....	2.01	—	—
5.....	50 cc. 2N sulphuric+10 gm. CaCO <sub>3</sub> .....	1.83	—	—
6.....	10 cc. 2N lactic .....	5.70	+	+
7.....	20 cc. 2N lactic .....	4.50	—	—
8.....	50 cc. 2N lactic .....	4.06	—	—
9.....	50 cc. 2N lactic+10 gm. CaCO <sub>3</sub> .....	7.03	+	+
10.....	10 cc. 2N lactic .....	6.46	+	+
11.....	20 cc. 2N lactic .....	5.09	+	+
12.....	50 cc. 2N lactic .....	3.73	—	—
13.....	50 cc. 2N lactic+10 gm. CaCO <sub>3</sub> .....	6.00	+	—
14.....	10 cc. 2N formic .....	6.20	+	+
15.....	20 cc. 2N formic .....	4.09	—	—
16.....	50 cc. 2N formic .....	3.43	—	—
17.....	50 cc. 2N formic+10 gm. CaCO <sub>3</sub> .....	7.78	+	—
18.....	10 cc. 2N citric .....	4.60	+	+
19.....	20 cc. 2N citric .....	2.62	—	—
20.....	50 cc. 2N citric .....	2.10	—	—
21.....	50 cc. 2N citric+10 gm. CaCO <sub>3</sub> .....	2.55	—	—

<sup>a</sup> + = Presence of Azotobacter

— = Absence of Azotobacter

Experiment set up Aug 6 Moisture content optimum

Acidity determined electrometrically

The data presented in Table VII indicate that if sufficient calcium carbonate is added to neutralize the added acid the Azotobacter will not be affected. However, large quantities of calcium carbonate are ineffective unless the quantity is sufficient to maintain a favorable reaction. \*

#### CONCLUSIONS

(1) If two soils, one more acid than P<sub>H</sub> 6.0 and containing no Azotobacter and the other less acid than P<sub>H</sub> 6.0 and containing Azotobacter, are mixed in varying proportions, incubated for some time, and cultured for Azotobacter, all mixtures giving an acidity less than P<sub>H</sub> 6.0 will show the presence of Azotobacter, while all cultures very much more acid than P<sub>H</sub> 6.0 will fail to show Azotobacter.

(2) If sufficient calcium carbonate is added to a soil more acid than P<sub>H</sub> 6.0 and not containing Azotobacter to reduce the hydrogen-ion concentration to less than P<sub>H</sub> 6.0, the soil will support Azotobacter.

(3) If sufficient acid is added to a soil less acid than P<sub>H</sub> 6.0 and containing Azotobacter to increase the acidity to a point very much greater than P<sub>H</sub> 6.0, Azotobacter will disappear from the soil, provided this acidity exists for sufficient time to complete their destruction.

## OXIDATION OF SULPHUR BY MICROORGANISMS IN BLACK ALKALI SOILS<sup>1</sup>

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The accumulation of sodium carbonate in the soil (black alkali soil) brings about a condition in which the soil practically has to be abandoned as far as utilization for the growth of plants is concerned. Irrigation, whereby the carbonates are washed out from the soil, brings about a temporary and unsatisfactory relief. If the sodium carbonate could be converted into sodium sulphate (white alkali), much more satisfactory results could be obtained, since, as pointed out recently by Vinson and associates (13),<sup>2</sup> white alkali is readily and completely leached out from soil, while black alkali resists leaching. The use of sulphuric acid on alkali soil has been suggested by C. B. Lipman and Sharp (6); this acid was found to exert a favorable influence upon the soil by neutralizing the carbonate and improving the physical condition of the soil through flocculating the colloids. However, the sudden introduction of large quantities of acid into the soil may have an injurious influence upon the soil microflora. Elementary sulphur would prove, in this respect, of greater benefit, since, not only would the injury to the soil microflora be less but the addition of small quantities of sulphur even may stimulate bacterial activities.

J. G. Lipman (7) was the first to suggest the use of sulphur for alkali soil, in order that the acid formed from the oxidation of sulphur may reduce the alkalinity of the soil and transform the sodium carbonate into sodium sulphate. O'Gara (9), when applying sulphur and sulphuric acid to soils, observed that there was a reduction of the carbonate and an increase in the sulphate content of the soil and a decided increase in the crop yield. Hibbard (3) demonstrated that by adding sulphur to alkali soil the alkalinity is neutralized, this effect being of great value in the reclamation of alkali land. Rudolfs (10) found on adding elemental sulphur, at the rate of 1,000 pounds per acre, that the reaction of the soil was reduced from  $P_H$  between 9.6 and 9.8 to  $P_H$  9.3; with 2,000 pounds per acre, to  $P_H$  9.2; with 3,000, to  $P_H$  8.9; and with 3,500, to  $P_H$  8.2. However, if black alkali soil is first leached, the use of 3,000 pounds of sulphur per acre will change the reaction of the soil from  $P_H$  9.2 to  $P_H$  7.7.

Although some of the sulphur may be oxidized in the soil without the intervention of life, as pointed out by Kappen and Quensell (5) and others, it is primarily as a result of activities of certain microorganisms that the rapid oxidation of the elemental sulphur takes place in both

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 305.

acid and alkaline soils. Two organisms have been isolated, which are responsible for this process. These two organisms are different in nature, in both their morphological and physiological characters: (1) *Thiobacillus thiooxidans*, isolated at this station from sulphur-soil composts and described in detail elsewhere (15), was found to be very active in oxidizing sulphur practically quantitatively in acid soils; and (2) *Thiobacillus B* was isolated from alkaline soils on which the sulphur had been actively oxidized. The organism resembles in general morphological and physiological characteristics *T. thioparus*, studied by Nathanson (8) Beijerinck (1), and others, and occurs commonly in the soil. It grows readily in alkaline media having a reaction equivalent to  $P_H$  9.8 and oxidizes readily thiosulphate with an abundant formation of elementary sulphur. A detailed description of methods for the study of this organism and its relation to the oxidation of sulphur in the soil are published elsewhere (14). No definite proof can, however, be given that *T. B* is absolutely pure and not contaminated with *T. thiooxidans* or a related strain.

In the experiment reported below, a black alkali soil from the University of California Ranch near Fresno, Calif., was used. Dr. Hoagland, of the University of California, who kindly supplied the soil, stated that "the physical condition of the soil was bad, the soil was extremely alkaline, and no ordinary growth of plants is supported." The reaction of the soil was about  $P_H$  9.6 to 9.8.

The soil was placed in 100-gm. portions in tumblers and the proper amount of sulphur was added and thoroughly mixed with the soil. Fifty mgm. of sulphur in 100 gm. of soil is equivalent to 1,000 pounds of sulphur per 1 acre of soil (2,000,000 pounds per acre basis). The proper quantity of water was added and the tumblers were incubated at 25° to 28° C. in the dark.

The reaction of the soil was determined by shaking thoroughly 5 gm. of soil with 10 cc. of water, centrifuging, then determining the hydrogen-ion concentration ( $P_H$  value) colorimetrically and in some cases electrometrically. The sulphates were determined by shaking the soil with distilled water (2) in a shaking machine for 2 to 6 hours, then filtering clear and precipitating as barium sulphate. The carbonates and bicarbonates were determined by the method outlined by Schreiner and Failyer (11).

Tables I and II show the course of reaction resulting from the oxidation of sulphur in alkali soil and the chemical transformations that have taken place as a result of these changes.

TABLE I.—Course of sulphur oxidation in alkali soil

Period of incubation.	No sulphur added.	50 mgm. sulphur per 100 gm. soil	100 mgm sulphur per 100 gm soil	200 mgm sulphur per 100 gm soil.	500 mgm. sulphur per 100 gm. soil.	1,000 mgm. sulphur per 100 gm soil.
	$P_H$	$P_H$	$P_H$	$P_H$	$P_H$	$P_H$
20 days. . . . .	9.6	9.6	9.1	9.0	8.9	8.7
42 days. . . . .	9.6	9.4	9.0	8.5	7.1	6.7
62 days. . . . .	9.6	9.4	8.5	7.8	7.6	7.0
86 days. . . . .	9.6	9.3	8.6	8.0	7.2	6.8
105 days. . . . .	9.6	9.1	7.9	7.2	6.0	4.4
167 days. . . . .	9.6	7.9	7.4	6.7	5.7	3.5

TABLE II.—Transformation of a black alkali soil into a white alkali<sup>1</sup>

	No sulphur added.		50 mgm. sulphur per 100 gm. soil.		100 mgm. sulphur per 100 gm. soil.		200 mgm. sulphur per 100 gm. soil.		500 mgm. sulphur per 100 gm. soil.		1,000 mgm. sulphur per 100 gm. soil.	
	Individual tumblers	Average	Individual tumblers	Average	Individual tumblers	Average	Individual tumblers	Average	Individual tumblers	Average	Individual tumblers	Average
P <sub>H</sub> .....	9.6	9.6	$\left\{ \begin{array}{c} 7.8 \\ 8.0 \\ 7.9 \end{array} \right\}$	7.9	$\left\{ \begin{array}{c} 7.6 \\ 7.1 \\ 7.4 \end{array} \right\}$	7.4	$\left\{ \begin{array}{c} 6.4 \\ 7.0 \\ 6.6 \end{array} \right\}$	6.7	$\left\{ \begin{array}{c} 5.5 \\ 5.5 \\ 6.2 \end{array} \right\}$	5.7	$\left\{ \begin{array}{c} 2.8 \\ 3.0 \\ 4.6 \end{array} \right\}$	3.5
Sulphates (milligrams of S in 100 gm. of soil).....	$\left\{ \begin{array}{c} 26 \\ 26 \\ 29.5 \end{array} \right\}$	27.2	$\left\{ \begin{array}{c} 77.6 \\ 72.4 \\ 77.5 \end{array} \right\}$	75.8	$\left\{ \begin{array}{c} 94.5 \\ 95.5 \\ 93.6 \end{array} \right\}$	94.9	$\left\{ \begin{array}{c} 158.0 \\ 172.8 \\ 162.4 \end{array} \right\}$	164.4	$\left\{ \begin{array}{c} 229.3 \\ 225.5 \\ 188.6 \end{array} \right\}$	211.1	$\left\{ \begin{array}{c} 636.3 \\ 436.7 \\ 270.0 \end{array} \right\}$	447.7
Percentage of sulphur oxidized.....	.....	.....	.....	97.2	.....	67.7	.....	67.6	.....	36.8	.....	42.05
Carbonates (milligrams of CO <sub>2</sub> in 100 gm. of soil).....	$\left\{ \begin{array}{c} 47.2 \\ 51.2 \\ 49.2 \end{array} \right\}$	49.2	$\left\{ \begin{array}{c} 7.9 \\ 5.9 \\ 5.9 \end{array} \right\}$	6.6	.....	0	.....	0	.....	0	.....	0
Bicarbonates (milligrams of HCO <sub>3</sub> in 100 gm. of soil).....	$\left\{ \begin{array}{c} 182 \\ 178 \\ 172 \end{array} \right\}$	177.3	$\left\{ \begin{array}{c} 102 \\ 104 \\ 104 \end{array} \right\}$	103.3	$\left\{ \begin{array}{c} 70 \\ 72 \\ 74 \end{array} \right\}$	72.0	$\left\{ \begin{array}{c} 38 \\ 32 \\ 34 \end{array} \right\}$	34.7	$\left\{ \begin{array}{c} 20 \\ 15 \\ 24 \end{array} \right\}$	20.0	$\left\{ \begin{array}{c} 0 \\ 0 \\ 10 \end{array} \right\}$	3.3

<sup>1</sup> Cultures incubated 167 days at 25° to 27° C

The results brought out in Tables I and II point out definitely, that not only is elemental sulphur oxidized readily to sulphuric acid, which results in the neutralization of the carbonates of the soil, but the reaction of the soil can be brought down to any desired point, depending entirely upon the quantity of sulphur added and the length of time during which the sulphur is allowed to be in contact with the soil.

The next experiment deals with the oxidation of sulphur by pure and crude cultures of the sulphur-oxidizing bacteria in alkaline soil under sterile conditions. The soil was placed in 100-gm. portions in 250 cc. Erlenmeyer flasks; 100, 200, and 500 mgm. portions of sulphur were added to the soil portions, which were then thoroughly mixed, then the optimum amount of water was added and the flasks were sterilized for 1½ hours at 15 pounds pressure. The flasks were then inoculated by means of a sterile pipette, with 2 drops of the vigorously growing cultures and incubated at 25° to 28° C. Alkaline soils in which active sulphur oxidation had taken place were used as the crude alkaline compost. The results are given in Table III.

TABLE III.—Oxidation of sulphur in alkaline soil by different bacteria

Quantity of sulphur used.	Type of culture.	Incubated 14 days.	Incubated 30 days.			Incubated 60 days.			
		P <sub>H</sub>	P <sub>H</sub>	Carbonates in 100 gm. of soil (mgm. of CO <sub>2</sub> ).	Bicarbonates in 100 gm. of soil (mgm. of HCO <sub>3</sub> ).	P <sub>H</sub>	Carbonates in 100 gm. of soil (mgm. of CO <sub>2</sub> ).	Bicarbonates in 100 gm. of soil (mgm. of HCO <sub>3</sub> ).	Sulphates in 100 gm. of soil (mgm. of S).
None.....	Control.....	9.6	9.6	49.0	159	9.6	52.5	143	30.1
100 mgm.....	Crude alkaline compost.....	7.7	7.5	0	50.0	7.7	0	43.5	115.5
200 mgm.....	do.....	7.4	6.3	0	31.0	5.6	0	8.5	201.0
500 mgm.....	do.....	7.0	4.9	0	15.0	4.7	0	6.5	254.8
100 mgm.....	<i>Thiobacillus thiooxidans</i> .....	9.8	8.8	8.0	142.0	8.6	0	86.0	109.4
200 mgm.....	do.....	8.6	9.0	14.7	127.0	7.5	0	53.0	145.3
500 mgm.....	do.....	8.6	9.0	5.8	118.0	7.4	0	45.0	160.7
100 mgm.....	<i>Thiobacillus B.</i> .....	8.8	9.0	29.4	132.0	8.8	0	80.0	78.2
200 mgm.....	do.....	8.2	8.0	18.5	138.0	8.0	0	55.5	118.4
500 mgm.....	do.....	8.0	7.8	15.6	136.0	7.6	0	48.0	123.3



The crude culture proved to be most efficient in oxidizing sulphur in alkaline soil to such an extent that the reaction has been reduced, where sufficient sulphur has been used, from most alkaline to distinctly acid. This was accompanied by a complete disappearance of the carbonates, an almost complete disappearance of the bicarbonates, and an increase in the quantity of sulphates. With 100 mgm. of sulphur per 100 gm. of soil, equivalent to 1 ton of sulphur per acre (on the basis of the upper  $6\frac{3}{4}$  inches of soil), 85 per cent of the sulphur has been oxidized by the crude culture to sulphates within 60 days, and the reaction reduced from  $P_H$  9.6 to  $P_H$  7.7. When the quantity of sulphur was doubled, practically the same percentage of sulphur (85.5) was oxidized in 60 days and the  $P_H$  changed to 5.6. Where a large excess of sulphur was used, or 500 mgm. per 100 gm. of soil, only 44 per cent of the sulphur was oxidized to sulphate, and the reaction of the soil changed to  $P_H$  4.7, which would already prove injurious to certain crops because of the excess acidity. The pure cultures did not prove quite as effective as the crude culture. This may be due to the fact that the oxidation of sulphur in alkaline soil is carried out not by one organism but by several organisms taking part in the process, as is shown by the experiments reported in Table IV, where a mixture of the two organisms is used. The acid soil used in these experiments is a Sassafra sandy loam, slightly acid in reaction. These experiments were carried out both under sterile and nonsterile conditions in tumblers.

TABLE IV.—Oxidation of sulphur in acid and alkaline soils by pure and crude cultures of sulphur-oxidizing bacteria

Soil type	Sterilization of soil. <sup>1</sup>	Organism.	Incubated 21 days.	Incubated 48 days
			$P_H$	$P_H$
Acid; 100 gm. of soil + 100 mgm. of sulphur.	+	Control.....	6.0	5.8
	—	do.....	5.2	4.4
	+	Crude, acid compost....	5.7	3.8
	—	do.....	4.8	3.8
	+	Crude, alkaline compost....	5.4	5.0
	—	do.....	5.4	4.0
	+	<i>Thiobacillus thiooxidans</i> ....	5.6	5.0
	—	do.....	5.0	3.8
	+	<i>Thiobacillus B.</i> .....	5.8	5.4
	—	do.....	5.4	4.0
	+	<i>Thiobacillus thiooxidans</i> + <i>Thiobacillus B</i> .....	5.4	3.4
	—	do.....	4.8	3.6
Alkali; 100 gm. of soil + 200 mgm. of sulphur.	+	Control.....	9.6	9.2
	—	do.....	8.8	8.2
	+	Crude, acid compost....	9.0	8.2
	—	do.....	9.0	8.4
	+	Crude, alkali compost.....	7.5	7.1
	—	do.....	7.6	7.3
	+	<i>Thiobacillus thiooxidans</i> ....	8.8	8.6
	—	do.....	8.2	8.0
	+	<i>Thiobacillus B</i> .....	8.2	7.8
	—	do.....	8.0	7.6
	+	<i>Thiobacillus thiooxidans</i> + <i>Thiobacillus B</i> .....	7.8	7.6
	—	do.....	7.6	7.4

<sup>1</sup> + indicates that the flasks containing soil and sulphur were sterilized, at 15 pounds pressure, for 1½ hours; — = soil unsterilized, in tumblers.

In the acid soil, oxidation of sulphur took place in the unsterilized containers whether inoculated or uninoculated, more so in the inoculated cultures, particularly where the *Thiobacillus thiooxidans* was introduced. This is due to the fact that the sulphur added, in nonsterile condition, probably has been inoculated previously from the laboratory air. However, when the soil and the sulphur are previously sterilized, oxidation takes place only to a very unappreciable extent, unless the proper sulphur-oxidizing organisms are introduced. In the alkaline soil, the most efficient results were obtained from inoculation with the crude alkaline culture, or alkaline soil, in which sulphur oxidation had taken place previously. The pure cultures, particularly the *Thiobacillus B* culture, which was almost inactive in the acid (sterilized) soil, proved more efficacious than the *T. thiooxidans*; but a mixture of the two cultures proved nearly as efficacious as the crude alkaline composts.

The fact that the change of the soil carbonates to sulphates would also favor plant growth, which is found to be so in actual field results, was demonstrated in these experiments by the increase in the number of bacteria developing in the soil. The soils used in the experiment reported in Tables I and II were at the end of the experiment air dried, then again moistened and, after 5 to 6 days, the bacterial numbers were determined by the ordinary plate method. The alkali soil, to which no sulphur had been added, contained 320,000 bacteria per gram of soil; where 50 mgm. of sulphur had been added per 100 gm. of soil and the reaction changed to  $P_H$  7.9, there were 665,000 bacteria per gram; 100 mgm. of sulphur, reaction  $P_H$  7.4, 875,000; 200 mgm. of sulphur, reaction  $P_H$  6.7, 1,275,000; 500 mgm. of sulphur, reaction  $P_H$  5.7, 3,650,000; 1,000 mgm. of sulphur, reaction  $P_H$  3.5, no bacteria, only a few occasional fungi. In this case, the soil reaction has been made so acid as to completely kill off the bacteria. This danger, however, could hardly be expected in the field, because no such large quantities of sulphur (1 per cent) would ever be employed.

In the following experiment, the reaction of the soil was adjusted by the addition of sulphuric acid and sodium carbonate. The following amounts of acid and alkali were used to adjust the reaction of the soil:

	$P_H$ .
Untreated soil . . . . .	5.6
5 cc. $N/2 H_2SO_4$ per 100 gm. of soil . . . . .	5.3
0.5 cc. $M/2 Na_2CO_3$ per 100 gm. of soil . . . . .	5.8
1.0 cc. $M/2 Na_2CO_3$ per 100 gm. of soil . . . . .	6.0
3.0 cc. $M/2 Na_2CO_3$ per 100 gm. of soil . . . . .	6.2
7.0 cc. $M/2 Na_2CO_3$ per 100 gm. of soil . . . . .	6.4
20.0 cc. $M/2 Na_2CO_3$ per 100 gm. of soil . . . . .	8.4

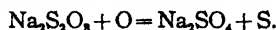
One-gm. portions of powdered sulphur were added to each 100-gm. portion of soil in Erlenmeyer flasks; the flasks were well shaken, plugged with cotton, sterilized at 15 pounds pressure for  $1\frac{1}{2}$  hours, then inoculated with pure cultures of the organisms and incubated at  $25^\circ C$ . At the end of definite intervals of time the moisture content was brought up by the addition of sterile distilled water. The results are presented in Table V.

TABLE V.—Oxidation of sulphur by pure cultures of bacteria at various reactions

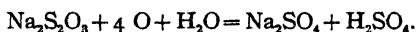
Type of culture.	P <sub>H</sub> value.				Soluble sulphates (sulphur) in 100 gm. of soil after 50 days' incubation.
	Initial reaction of soil.	16 days after.	30 days after.	50 days after.	
					Mgm.
Control.....	5.2	5.2	5.3	5.4	19.2
	5.6	5.6	5.6	5.6	13.7
	5.8	5.8	5.8	5.7	11.0
	6.0	6.0	5.8	5.8	13.7
	6.2	6.2	6.2	6.2	13.7
	8.4	8.4	8.2	8.2	21.9
	5.2	5.2	3.4	2.8	315.1
	5.6	5.4	3.0	2.8	314.6
<i>Thiobacillus thiooxidans</i> .....	5.8	5.6	5.5	5.5	20.6(?)
	6.0	5.8	3.0	2.6	358.0
	6.2	5.4	2.8	2.8	380.0
	6.6	6.6	6.6	3.0	304.4
	8.4	8.4	8.2	8.0	27.4
	5.2	5.3	5.2	5.4	39.7
	5.6	5.3	5.2	5.2	20.6
	5.8	5.5	5.5	5.4	21.9
<i>Thiobacillus B</i> .....	6.0	6.0	5.5	5.5	15.2
	6.2	6.0	5.8	5.4	19.2
	6.6	6.6	6.6	6.4	24.7
	8.4	8.2	8.0	7.8	.....
	5.2	5.2	3.4	2.8	302.5
	5.6	3.1	2.8	2.6	287.7
	5.8	2.8	2.8	2.6	311.1
	6.0	2.8	2.6	2.6	308.3
<i>T. thiooxidans</i> + <i>T. B</i> .....	6.2	2.8	2.8	2.6	367.0
	6.6	3.1	2.8	2.8	274.0
	8.4	8.2	8.0	7.4	.....

Both the reaction and the sulphur content of the control indicate that no sulphur or only traces of it were oxidized under sterile conditions. However, in the presence of the sulphur-oxidizing bacteria, the oxidation of the sulphur took place very rapidly, with the transformation of large quantities of sulphur to sulphuric acid. When the reaction of the soil was acid, the oxidation of the sulphur in 50 days was carried on entirely by *Thiobacillus thiooxidans*. *Thiobacillus B* oxidized only very small quantities of sulphur; but, in the presence of both organisms, the speed of the reaction was hastened, as indicated by the measurements of the P<sub>H</sub> values obtained in 16 days. This may be explained by either of two assumptions: (1) If we suppose a so-called "lag phase" in bacterial development, *Thiobacillus B* produces substances which appreciably shorten the "lag phase" of *T. thiooxidans*; (2) *Thiobacillus B* may oxidize the sulphur not directly to sulphates but to other compounds of sulphur, which are then rapidly oxidized to sulphates by *T. thiooxidans*. This is made clear in the study of the pure cultures of *Thiobacillus B*, with sodium thiosulphate as a source of energy, when a large part of the thiosulphate is transformed to persulphates, a part to elementary sulphur, and only a part to sulphates. This preparatory function of the *Thiobacillus B* may explain a number of hitherto unexplained difficulties observed in the study of sulphur oxidation by microorganisms in the soil. Of these we need mention only a few.

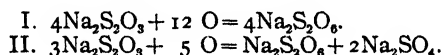
Nathanson (8), who first studied the *Thiobacillus* group, of which *Thiobacillus B* (not *T. thiooxidans*) is a representative, stated that thiosulphate is oxidized to sulphate and tetrathionate, with the production of free sulphur; however, on sodium sulphid agar plates, when free sulphur is formed abundantly in a nonbiological way, this sulphur is oxidized by the bacteria. The reaction proposed by Nathanson ( $3\text{Na}_2\text{S}_2\text{O}_3 + 5\text{O} = \text{Na}_2\text{S}_4\text{O}_6 + 2\text{Na}_2\text{SO}_4$ ) could hardly be justified, particularly in view of the fact that the sulphur was supposed to be produced in a purely nonbiological way, by the interaction of the tetrathionate with the remaining thiosulphate, and was not supposed to play any part in the reaction. Beijerinck (1), who identified Nathanson's organism with his *T. thioparus*, then suggested the following reaction:



But Jacobsen (4), who worked in Beijerinck's laboratory, found that the same organism oxidized elemental sulphur quantitatively to sulphate, which would again hardly justify Beijerinck's formula, if the organism was the same. Trautwein (12), however, obtained the separation of elemental sulphur from thiosulphate only in crude cultures of the organisms; but, in pure culture, no separation of elemental sulphur was found. At the same time a large part of the thiosulphate was transformed to sulphate. At first he thought the following reaction to be justified:



In view of the fact that the reaction of the medium did not turn acid and that a large quantity of persulphate was formed, he finally concluded that the reaction takes place as follows:



In the investigations carried on in this laboratory with pure cultures of *Thiobacillus B*, it was found that this organism transforms thiosulphate into persulphate, sulphate, and elemental sulphur, with only a limited production of acid ( $P_H$  changed in culture solution from 9.8 to 6.4 or 7.0). When *T. thiooxidans* is added to the culture solution, the elemental sulphur separated by the *Thiobacillus B* is rapidly oxidized to sulphuric acid and the final reaction may go down from  $P_H$  9.8 to  $P_H$  1.2 as a result of the action of the two organisms. The culture solution used in this case consists of 5 gm. sodium thiosulphate, 1 gm. sodium bicarbonate, 0.2 gm. dipotassium phosphate, 0.1 gm. magnesium chlorid, 0.1 gm. ammonium chlorid, 0.25 gm. calcium chlorid, and 1,000 cc. of tap water.

These discrepancies in the type of reaction that takes place when thiosulphate is acted upon by sulphur-oxidizing bacteria can be explained by the fact that different workers used different forms of a closely related group of organisms; in some cases the culture used possibly contained not one organism but a mixture of two or more organisms. *Thiobacillus B*, isolated in this laboratory and closely related, in its morphology and physiology, to *T. thioparus* and allied forms studied by Nathanson (8), Beijerinck (1), Trautwein (12), and others (4), was possibly contaminated in some cases by *T. thiooxidans*, which oxidizes actively elemental sulphur and is described by Waksman and Joffe (15). *T. thioparus* will act upon sulphur compounds under distinctly alkaline conditions, as in

culture media containing soluble carbonates or bicarbonates or black alkali soil; the elemental sulphur will be oxidized only comparatively slowly, but, in the presence of *T. thiooxidans* this oxidation will be rapidly hastened and the reaction quickly changed to any degree of acidity, depending merely on the quantity of sulphur used.

The possibility of the participation of other organisms oxidizing sulphur or sulphur compounds in the soil, or in any way influencing the process, in addition to the two mentioned, is not excluded. Neither is the possibility excluded that the oxidation of sulphur in alkaline soil is carried on chiefly by a strain of *Thiobacillus thiooxidans* which has adapted itself to alkaline conditions. While *T. thiooxidans* grows vigorously on artificial media and does not deteriorate with age, *Thiobacillus B* rapidly deteriorates under cultural conditions and the very slow oxidation of sulphur by this organism, particularly in the latter part of the work, may be due to this phenomenon.

#### SUMMARY

(1) The use of sulphur in the presence of the proper sulphur-oxidizing bacteria will result in the transformation of black alkali soil to white alkali soil.

(2) The final reaction of the soil depends on the quantity of sulphur used and the length of time which the sulphur is allowed to be in contact with the soil.

(3) The oxidation of sulphur in black alkali soil is probably carried on by the agency of more than one sulphur-oxidizing bacterium.

(4) In the presence of two bacteria, one of which can act upon sulphur under distinctly alkaline conditions while the other rapidly oxidizes sulphur under acid conditions, the speed of the reaction is greatly hastened.

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# PEACH ROSETTE, AN INFECTIOUS MOSAIC<sup>1</sup>

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## HISTORY OF THE DISEASE

According to Smith<sup>2</sup> peach rosette was first noted in Georgia in 1881. By 1891 this disease had been reported from 22 counties in Georgia, from South Carolina, and from Kansas. Rosette was also reported as occurring on plums in Georgia and Kansas, and on almonds in Kansas.

Since 1903, reports of the presence of rosette have come to the Plant Disease Survey, of the United States Department of Agriculture, from 19 counties in Georgia, 1 county in Alabama, 5 counties in South Carolina, 4 counties in Tennessee, 1 county in West Virginia, 19 counties in Missouri, and 2 counties in Oklahoma. These records indicate that for the past 40 years rosette has taken its toll of trees, and that it has spread over a considerable area.

## IDENTITY OF THE DISEASE

In 1890-91, Smith<sup>2</sup> conducted experiments in middle Georgia in which he showed that peach rosette is an infectious disease. Of 125 seedling peach trees into which he inserted buds from a rosetted peach tree, 121 developed rosette.

In June, 1891, Smith<sup>2</sup> inoculated 37 Elberta peach trees with buds from a rosetted Kelsey plum. Two trees developed rosette and died in August, 1892. The other inoculated trees remained healthy. As a result of this experiment Smith said:

The small per cent of cases to unions makes it necessary to repeat this experiment before it can be stated positively that the plum disease is identical with that of the peach and transmissible to it, as seems very probable from its appearance.

In June, 1891, Smith<sup>2</sup> inoculated 104 Marianna plum trees by inserting buds from rosetted peach trees. After 16 months not a single case of rosette had developed on the Marianna plums, so he concluded:

There is, therefore, good reason to believe that the Marianna plum is not subject to this disease.

In June, 1891, 12 Marianna plum trees were inoculated by Smith<sup>2</sup> with buds from a rosetted Kelsey plum. On final examination in November, 1892, the Marianna plums showed no signs of rosette.

The presence of rosette in orchards at the Georgia Experiment Station, and in a number of commercial orchards in various sections of the State led to further study of this disease by the writer, beginning in 1919. As data presented by Smith<sup>4</sup> indicated that fungi and microscopic bacteria were not the cause of rosette, the writer did not attempt to repeat this phase of the work.

<sup>1</sup> Accepted for publication Aug. 18, 1922.

<sup>2</sup> SMITH, Erwin F. ADDITIONAL EVIDENCE ON THE COMMUNICABILITY OF PEACH YELLOWS AND PEACH ROSETTE. PART II. PEACH ROSETTE. In U. S. Dept. Agr. Div. Veg. Path. Bul. 1, p. 45-54. 1891.

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## PEACH TO PEACH, PLUM, AND APRICOT

EXPERIMENT 1.—In the spring of 1919, rosette appeared on one side of a six-year-old seedling peach tree in an experimental orchard at the station. The other side of this tree appeared normal throughout the summer of 1919. During this time buds from healthy peach, plum, and apricot trees were inserted into the new growth of the normal appearing branches. The plum buds failed to unite with the peach stock. The peach and apricot buds united with the peach stock, but remained dormant throughout the summer. In the spring of 1920, the branches of the seedling peach which showed rosette in 1919, did not produce leaves, and examination showed that they were dead. The branches which appeared normal in 1919 produced rosetted growth in the spring of 1920. The peach and apricot buds inserted in 1919 produced typical rosetted shoots showing that the causal entity had passed from the diseased peach stock to the buds. The apricot buds produced shoots from two to three inches in length and the leaves had the mottled appearance of a mosaic disease. The mottled appearance was not so striking on the peach leaves. The shortened internodal growth, together with mottling of the leaves of some hosts and the absence of microscopic bacteria, put peach rosette in the class of virus or mosaic diseases. This experiment indicated that both the peach and the apricot are hosts for rosette.

## PEACH TO PEACH

EXPERIMENT 2.—On June 10, 1919, buds from a healthy Elberta peach tree about 20 years of age were put in the new growth of two seedling peach trees about two years of age to serve as controls. On the same date buds from rosetted shoots of the seedling peach described in experiment 1 were put in the new growth of two peach seedlings the same age as the controls. On August 29, 1919, it was observed that the Elberta buds on the control trees had grown into healthy shoots. In one of the inoculated trees a diseased bud had developed a rosette of leaves, and below the point where this bud was inserted some of the buds of the stock had developed rosettes characteristic of this disease. In the other inoculated peach seedling the bud had remained dormant, but the buds of the stock immediately below the point of inoculation had developed typical rosettes. In May, 1920, it was observed that the two control trees were healthy and growing vigorously, while the inoculated trees had become completely rosetted (Pl. 1, A).

## PEACH TO APRICOT TO PEACH

EXPERIMENT 3.—On August 15, 1919, two buds from a rosetted twig of the peach tree described in experiment 1 were put into the new growth of a healthy Royal apricot, which was in its second year of growth. The peach buds remained dormant until the spring of 1920 when they developed into rosetted shoots. The lateral buds on the apricot branch below the point of inoculation also developed weak shoots, but the leaves did not have the typical rosetted appearance. The internodal growth, although longer than that of the rosetted peach shoot (Pl. 2, A), was much less than that of healthy apricot trees growing near. Plate 2, B, shows the stunted growth of this Royal apricot 12 months after inoculation with peach rosette. While the growth is not typical of rosette as

it appears in the peach, the shortened internodal growth, and the mottling of the leaves indicate that the causal entity had been transferred from the peach to the apricot.

On September 2, 1920, two buds were taken from the rosetted Royal apricot and inserted in a peach seedling, growing in a pot in the greenhouse. These buds remained dormant until April, 1921, when they began to develop mottled leaves. The new growth of the peach seedling developed typical rosettes (Pl. 1, B). Both the potted rosetted peach seedling and the rosetted Royal apricot tree died during the summer of 1921.

Experiment 3 shows that the causal entity of rosette may be transferred from peach to apricot, and from apricot to peach, proving that apricot and peach rosette are identical as far as causal entity is concerned, but somewhat different in external manifestations of the disease.

#### STUNTING OF APRICOT GROWTH BY ROSETTE

EXPERIMENT 4.—In the spring of 1920 another case of natural infection of peach rosette (Pl. 3, A) developed in a 6-year-old seedling peach tree growing in the same orchard about 150 feet from the rosetted seedling described in experiment 1.

On June 19, 1920, buds from this second rosetted seedling peach were inserted into a branch of a one-year-old Moorpark apricot about a foot above the ground. By August 20, 1920, both of the peach buds had grown into rosetted shoots (Pl. 5, B), but the stock showed symptoms of rosette only in the growth immediately below the inserted peach buds, where the apricot leaves became mottled. An apricot limb adjoining the one which was inoculated, made a growth of 4 feet and 4 inches during the summer of 1920, and bore normal green leaves. There was no growth of lateral buds. On April 11, 1921, the lateral as well as the terminal buds on all the apricot branches had produced mottled, greenish yellow leaves, in marked contrast to the normal green leaves of the near-by healthy apricot. This indicated that the causal entity of rosette had spread throughout the apricot tree. During the summer of 1921 this Moorpark apricot tree grew very slowly, the maximum growth of any one branch being 5 inches. During the same time the near-by healthy apricot tree made a growth of 4 feet and 9 inches. Plate 3, B (taken in the fall of 1921) shows the inoculated apricot tree with a background, at the left, in contrast to the healthy apricot tree at the right. An indication of the stunting effect of rosette is shown by the inoculated tree in which the maximum vertical growth of 4 feet and 4 inches made during the summer of 1920 represents normal growth; while the maximum horizontal growth, 5 inches, represents development made during the summer of 1921 under the retarding influence of rosette.

#### PEACH TO CULTIVATED PLUM

EXPERIMENT 5.—On August 6, 1919, a bud from a healthy Blue Damsion plum was placed in the trunk of a healthy peach seedling which came up in the spring of 1919. This plum bud made a few inches of growth during the summer of 1919, and in the summer of 1920, made a growth of more than 2 feet. Neither the plum branch nor the peach stock showed any symptoms of disease up to June 19, 1920, when two buds from the rosetted peach tree described in experiment 4, were inserted in

the Damson plum branch. The diseased peach buds started growth within two weeks, producing typical rosetted shoots, but with somewhat larger leaves and more internodal growth than did similar rosetted buds on the diseased peach stock from which the buds were taken. During the summer of 1920, the plum buds just below the place where the peach buds were inserted, developed small rosettes of mottled leaves (Pl. 4, A) indicating that the causal entity had passed from the diseased peach buds into the healthy Damson plum branch. None of the branches of the peach stock on which the plum was budded showed symptoms of rosette during the summer of 1920. The growth of the peach stock was so vigorous that a small copper wire, by which a label had been attached to the trunk in 1919, became embedded in the tissues of the stock, a few inches above the point where the Damson plum bud was inserted. In the spring of 1921, rosette developed in all growth of the Damson plum branch, and in all branches of the peach stock which grew from the trunk at points below where the copper wire was embedded in the tissues. None of these rosetted branches set fruit, though a few produced weak blossoms. All of the branches which grew from the trunk above where the wire was embedded produced normal leaves and blossoms which set numerous fruits. By July, 1921, the leaves on this tree began to wither, and by August 20 the tree was practically dead (Pl. 4, B). No symptoms of rosette appeared on the tree above the embedded wire, and the leaves and fruits shriveled and clung to these branches for some time after the tree was dead. The fact that no symptoms of rosette appeared above the embedded wire supplies additional data as to the tissues through which the causal entity progresses, and is being further investigated.

In this experiment rosette was transferred from the peach to the plum, and back to the peach, indicating that peach and plum rosette are identical.

Rosette has also been transferred from peach to Red June plum by means of infected buds.

#### PEACH TO WILD PLUM TO PEACH

EXPERIMENT 6.—On September 22, 1920, buds from a rosetted May-flower peach, 7 years old, which developed as a natural infection in one of the station orchards in the spring of 1920, were put in two wild Chickasaw plum trees, growing in a fence row on the station. These buds remained dormant until the spring of 1921, when both the peach buds and the plum stock developed rosetted shoots. The inoculated plums (Pl. 4, C) grew more slowly than the surrounding healthy plum trees during the summer of 1921. The appearance of the rosetted wild plum is not so striking as that of a rosetted peach, because the plum is naturally of dwarfed growth. The wild plum, being of no economic importance, grows in waste places without coming under the close observation of man; therefore one or more rosetted wild plum trees might easily be an unobserved source of infestation to surrounding orchards.

On May 23, 1921, buds from the rosetted wild Chickasaw plum were inserted in the new growth of a 2-year-old seedling peach. During the summer one of these plum buds produced a rosetted shoot about an inch in length, but no symptoms of rosette appeared in the peach stock up to the time it was defoliated by frost. In the spring of 1922 this

peach seedling showed rosette in all new growth (Pl. 5, A). Peach seedlings into which healthy plum buds were inserted in 1921 showed no symptoms of rosette in the new growth of 1922.

This indicates that the casual entity of rosette may readily be transmitted from peach to wild plum, and from wild plum to peach.

Rosette has also been transmitted from the wild Chickasaw plum to the Red June plum by means of infected buds.

#### PLUM TO PEACH

EXPERIMENT 7.—A natural infection of a Maynard plum developed in an orchard on the station in the spring of 1920. By June 15, 1920, most of the lateral buds had grown into rosetted shoots from 1 to 3 inches long; and by August, 1920, this tree (Pl. 6, B) had made very little new growth as compared with a near-by healthy plum tree (Pl. 6, A). On June 18, 1920, buds from this rosetted plum tree were put into a healthy seedling peach tree in its second season's growth. Buds from a healthy plum were put into another peach seedling to serve as a control. On August 18, 1920, it was observed that some of the rosetted Maynard plum buds put into the peach seedling had produced shoots several inches long. Below the point where the diseased buds were inserted the peach buds had developed rosetted shoots (Pl. 6, C). The disease continued to spread in this peach seedling during the rest of the season of 1920, and when new growth started in the spring of 1921 this tree showed rosette in all parts. It died before midsummer. One of the control buds produced normal leaves (Pl. 7, A) and the peach stock on which it was growing was alive and healthy in the fall of 1921 when it was removed to make room for other experimental work.

This experiment indicates that rosette originating in the cultivated plum may be transmitted to the peach.

#### PEACH TO MARIANNA PLUM

EXPERIMENT 8.—On June 18, 1920, buds from the rosetted peach seedling described in experiment 4 were put into a healthy Marianna plum branch, near the base of the tree. One bud started growth within two weeks and produced a rosetted shoot (Pl. 7, B) with larger leaves and longer internodal growth than rosetted shoots on peach stocks. No signs of rosette appeared on the Marianna stock during the summer of 1920. After becoming dormant in the fall of 1920, this Marianna plum was transplanted to a large pot and placed in the greenhouse. In the spring of 1921 the peach shoot developed rosetted leaves, but continued to grow throughout the summer. The Marianna plum stock developed normal leaves on all of its branches, which grew rapidly throughout the summer. This Marianna plum (Pl. 7, C) had been under observation in the greenhouse during the winter, and up to May 1, 1922, it showed no symptoms of rosette. The rosetted peach shoot continued to grow slowly. When rosetted peach buds were put into a susceptible host, as peach, apricot, or ordinary cultivated plums, they died within 12 months. On the resistant Marianna plum stock the rosetted peach bud grew into a shoot which at the time this paper was written had lived for 22 months. This indicated that the resistant stock exerted a marked influence on the virulence of the causal entity of rosette in the peach scion.

## PLUM TO MARIANNA PLUM

EXPERIMENT 9.—On June 18, 1920, buds from the rosetted Maynard plum described in experiment 7 were put into a Marianna plum branch, near the base of the tree. By September 18, 1920, one of the diseased buds had produced a rosetted shoot 8 inches in length with three branches from 3 to 5 inches in length (Pl. 8, A). This rosetted Maynard plum branch made considerably more growth on the Marianna stock than similar buds made on the susceptible Maynard stock, but the growth was decidedly rosetted and the leaves were mottled yellowish green. The Marianna stock grew vigorously throughout the summer of 1920 and showed no external symptoms of rosette.

Buds from a healthy Mayflower peach tree were put into the new growth of the Marianna stock on which the rosetted Maynard plum shoot was growing on September 18, 1920. These peach buds remained dormant until the spring of 1921, when two buds grew into healthy peach shoots. The Marianna plum branches and the Mayflower peach shoots made a vigorous growth during the summer of 1921, which showed no symptoms of rosette. The Maynard plum shoot made some growth during the summer of 1921, but at all times it had the characteristic symptoms of rosette. In the spring of 1922 the Marianna plum stock and the two Mayflower peach shoots (Pl. 8, B) developed normal leaves in contrast to the rosetted Maynard plum branch.

This experiment gave additional evidence that the Marianna plum is not susceptible to rosette. It also indicated that the causal entity of rosette does not pass from a host, such as the Maynard plum, through the tissues of the resistant Marianna plum stock to another susceptible host, as the Mayflower peach.

## PEACH TO MAZZARD CHERRY

EXPERIMENT 10.—On June 19, 1920, buds from the rosetted peach tree described in experiment 4 were put into a healthy Mazzard cherry seedling about 1 year of age. On the same date buds from a healthy Elberta peach tree were put into a near-by Mazzard cherry tree of the same age to serve as a control. One of the rosetted buds united with the cherry stock and made a very feeble growth of rosetted leaves. The healthy buds united with the cherry stock but remained dormant. During the summer of 1920 the inoculated cherry stock grew slowly as compared with the control tree. The leaves of the inoculated tree became yellowish green and the new growth was small and in tufts similar to rosettes of peach leaves. The inoculated cherry stock developed leaves from both lateral and terminal buds in the spring of 1921, giving the new growth a decidedly rosetted appearance, especially at the tips of the branches. The control cherry stock produced vigorous new growth from the terminal buds. Very little growth was made by the diseased cherry tree during the summer of 1921, as shown by the smaller and more rolled leaves compared to those of the healthy control tree. The healthy Mazzard cherry control tree matured its buds and became dormant during the fall of 1921, while the diseased cherry tree attempted to make new growth from the terminal buds throughout the winter. By April 25, 1922, the inoculated tree (Pl. 9, A) was much stunted and had the appearance of being in an advanced stage of rosette, while the control

tree (Pl. 9, B) showed no symptoms of rosette and had made a vigorous growth.

This experiment indicates that the causal entity of peach rosette may be transmitted to Mazzard cherry and may produce symptoms similar to but not exactly the same as rosette of the peach. In the case of the Mazzard cherry there is evidently some resistance to the causal entity of rosette, for the infected cherry tree was alive June 1, 1922 (when this paper was written), 23 months after showing symptoms of rosette. Rosette has also been transmitted to two additional Mazzard cherry trees by means of infected peach buds.

#### WILD PLUM TO BITTER ALMOND

EXPERIMENT 11.—Through the courtesy of members of the California Agricultural Experiment Station, fresh seed of Bitter almond, and Texas Seedling almond were obtained and planted in the greenhouse on November 24, 1920. During April, 1921, some of the young almond trees were transplanted to the nursery. On May 23, 1921, buds from a rosetted wild Chickasaw plum (used in experiment 6) were put into two Bitter almond seedlings in the nursery. Three uninoculated trees of the same variety served as control. The plum buds united with the almond stocks and during the summer of 1921, one grew into a rosetted shoot about 6 inches long. Almond buds on the stock below the point where the rosette plum buds were inserted grew into small rosetted shoots which died during the winter. By May 24, 1922, the new growth of this inoculated tree was stunted (Pl. 10, A), and the leaves were yellowish green. The other inoculated tree had shown no marked symptoms of rosette at the time this paper was written. The adjoining uninoculated trees made a vigorous growth in the spring of 1922 and showed no symptoms of rosette.

This experiment indicates that the Bitter almond is susceptible to rosette.

#### APRICOT TO BITTER ALMOND AND TEXAS SEEDLING ALMOND

EXPERIMENT 12.—On April 18, 1921, buds from the rosetted Royal apricot of experiment 3 were put into one Bitter almond and one Texas Seedling almond growing in pots in the greenhouse. The buds united with the almond stocks, but made very little growth during the summer of 1921. Lateral buds on the two almond stocks, below the points of inoculation, developed small rosetted shoots indicating that the causal entity had been transferred from the rosetted apricot to both Bitter almond and Texas Seedling almond. Uninoculated almond trees of the two varieties growing in near-by pots remained healthy. In the spring of 1922 the inoculated trees became rosetted in all parts; the growth was stunted and the leaves were yellowish green. The uninoculated trees made a vigorous growth, however, and bore healthy green leaves.

This experiment proves that both the Bitter almond and the Texas Seedling almond are susceptible to rosette.

#### ALMOND TO PEACH

EXPERIMENT 13.—On February 2, 1922, buds were taken from the rosetted Bitter almond and Texas Seedling almond of experiment 12 and inserted in healthy peach seedlings growing in pots in the greenhouse.

One of the Bitter almond buds had produced a much branched shoot about 16 inches long by June 1, 1922, the leaves of which were more tufted in growth than those of healthy Bitter almonds. The peach shoots which grew from the stock below the point of inoculation had developed an upward rolling of their older leaves and most of the lateral buds had produced small rosettes of yellowish green leaves (Pl. 10, B).

The Texas Seedling almond buds remained dormant and up to June, 1922, the peach stock into which they were inserted had shown no symptoms of rosette. Peach seedlings budded to healthy almonds have remained healthy up to June 1, 1922.

This, together with the foregoing experiments, indicates that peach, apricot, plum, and almond are susceptible to rosette and that in all cases the causal entity is the same.

#### SOIL TRANSMISSION OF ROSETTE

EXPERIMENT 14.—Natural infection of rosette has been observed by the writer in Georgia on peach trees from 2 to 8 years of age. Where rosette develops in an orchard of young trees the question arises as to the advisability of setting a healthy tree in the place from which a diseased tree has been removed. It has been proved by Smith<sup>5</sup> that infection may be produced through inoculation of peach roots, so it seemed advisable therefore to test soil transmission.

Two 6-year-old peach trees, which had developed rosette in the spring of 1919, were dug up September 5 of the same year and removed from the orchard. Early in January, 1920, a healthy 1-year-old peach tree on peach stock was set in each hole. No attempt was made to remove fragments of roots left from the rosetted trees. The two transplanted trees made satisfactory growth during the summers of 1920 and 1921 showing no symptoms of rosette. This indicates that one may safely set a healthy tree in a place from which a rosetted tree has been removed.

#### THE TRANSMISSION OF ROSETTE BY MEANS OF SAP FROM DISEASED TREES

In rosette of the several species of *Prunus* there is a shortening of internodal growth and in some cases mottling of the leaves similar to mosaic diseases of vegetable and field crops. Numerous inoculations have been made in various parts of susceptible species of *Prunus* using methods known to be successful in transmitting mosaics of other plants. In no case has rosette been produced by transfers of sap from rosetted to healthy trees, but experiments along this line are being continued. The results obtained thus far confirm data presented by Smith<sup>6</sup> to the effect that under artificial conditions rosette is transmitted only when an organic union takes place between infected tissues, and tissues of a susceptible host. Thus in experiments conducted to date, rosette differs from other mosaics in the method of artificial transmission, indicating that the causal entity is somewhat different from that of other mosaics.

<sup>5</sup> Smith, Erwin F. ADDITIONAL NOTES ON PEACH ROSETTE. In *Jour. Mycol.*, v. 7, p. 226-232. 1893.

<sup>6</sup> Smith, Erwin F. THE PEACH ROSETTE. In *Jour. Mycol.*, v. 6, p. 143-148, pl. 8-13. 1891.

## NATURAL TRANSMISSION OF ROSETTE

The development of rosette in trees, often at a considerable distance from any known source of infection, indicates that winged insects or birds may be associated with natural transmission of this disease.

Various insects are found associated with rosetted trees, the most abundant being the black peach aphid, *Anuraphis persicae niger* Smith. Numerous tests have been made by removing insects, including several species of beetles and leafhoppers from various parts of rosetted trees, and caging them on healthy peach and plum trees. In no case has rosette developed. At various times throughout the growing season for the past two years, numerous black peach aphids have been transferred from rosetted peach and plum trees to healthy peach, plum, cherry, apricot, and wild plum trees growing in cages. The colonies of aphids increased rapidly in size showing that they were under favorable conditions. In no case did rosette develop as a result of these aphid transfers. These tests indicate that the causal entity of rosette is not readily transferred by the types of insects which are known to carry mosaic virus of other plants.

The writer is of the opinion that further study will disclose the fact that an animal, other than man, is responsible for the dissemination of the causal entity of rosette. Therefore observations along this line are being continued.

## SUMMARY

The data obtained from the foregoing experiments verify the findings of Smith to the effect that rosette is readily transmitted from peach to peach by infected buds.

Rosette has also been transmitted to two varieties of apricots, two varieties of cultivated plums, one wild plum, one cherry, and two varieties of almonds by means of infected buds.

On some hosts rosette produces a mottling of the leaves similar to mosaics.

The Marianna plum is immune to rosette.

Limited tests indicate that rosette is not soil-transmitted.

Numerous attempts to transmit rosette by means of sap from diseased trees has proved unsuccessful.

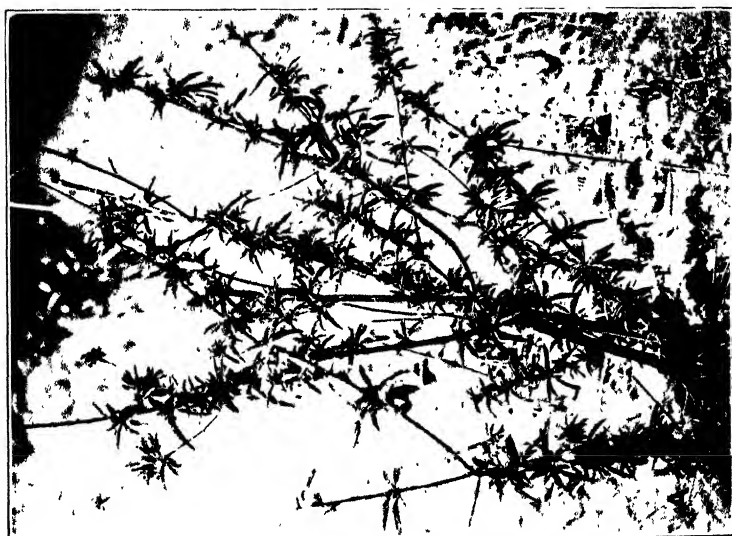
In a large number of transfers of various types of insects from rosetted trees to healthy susceptible hosts, not a single case of rosette was transmitted.

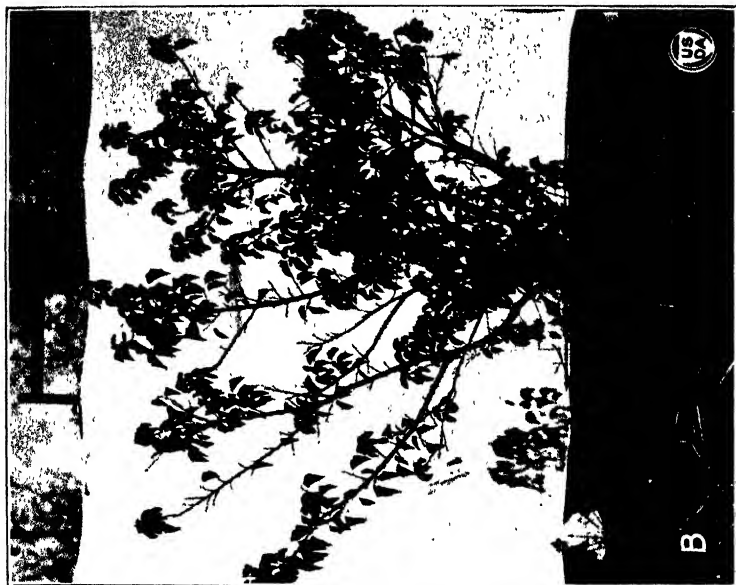


**PLATE 1**

A.—A 2-year-old peach tree into which buds from a rosetted peach were inserted June 10, 1919. Note the appearance in May, 1920, with all growth rosetted.

B.—The peach seedling into which buds from a rosetted Royal apricot were inserted September 2, 1920. Note the appearance in the summer of 1921, with all growth rosetted.





### **PLATE 2**

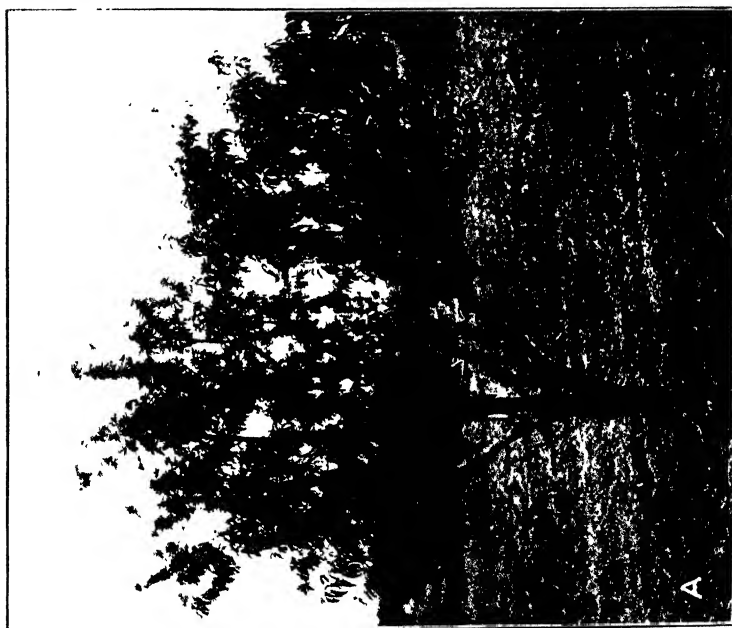
A.—A Royal apricot tree into which buds from a rosetted peach were inserted August 15, 1919. Note the rosetted peach shoot, and the stunted growth of the apricot shoots, made in 1920.

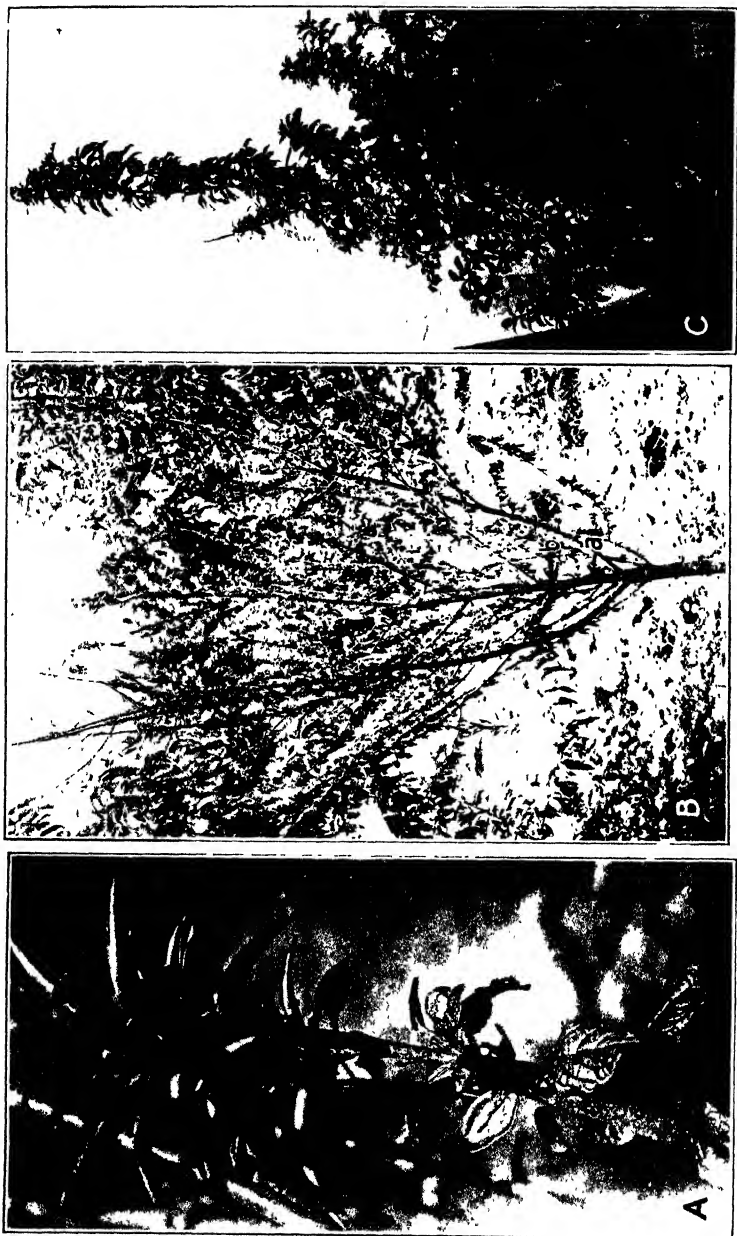
B.—The Royal apricot tree inoculated with peach rosette August 15, 1919. Note the stunted appearance of the whole tree on September 2, 1920.

### PLATE 3

A.—A 6-year-old peach tree which developed a natural infection of rosette in the spring of 1920. Note the rosetted growth on the left in comparison with the apparently normal growth of two limbs on the right. During the summer of 1920 this tree developed symptoms of rosette in all parts.

B.—To the left, with a background, the Moorpark apricot shown in Plate 5, B, which was inoculated with peach rosette, June 19, 1920. Note the stunted growth of this tree in the fall of 1921, in comparison with the healthy apricot tree, to the right, without a background.





#### PLATE 4

A.—The Blue Damson plum branch into which rosetted peach buds were inserted June 19, 1920. Note the rosetted and mottled plum leaves just below the rosetted peach shoots.

B.—The peach seedling which was infected with rosette through a Blue Damson plum branch inoculated with buds from a rosetted peach. The arrow at *a*, shows the point on the peach stock from which the plum branch grew from a healthy bud inserted in 1919. The arrow at *b*, shows the location of the embedded copper wire above which the causal entity of rosette did not go. Note the condition August 20, 1920, with the leaves and fruits shriveled and dying.

C.—One of the wild Chickasaw plum trees into which rosetted Mayflower peach buds were inserted September 22, 1920. Note the stunted growth and the rosetted condition of this plum tree on September 2, 1921.

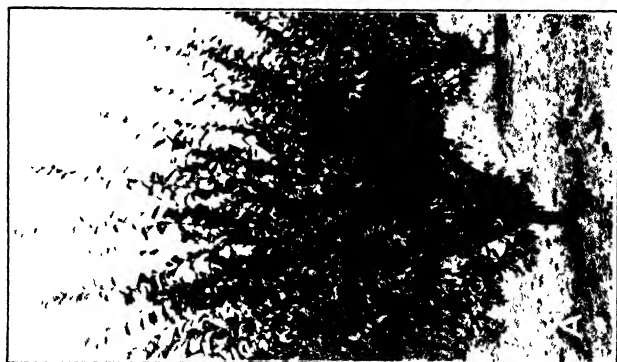


PLATE 5

A.—A seedling peach into which buds from a rosetted wild Chickasaw plum were inserted May 23, 1921. Note the completely rosetted condition of the peach seedling in May, 1922.

B.—A Moorpark apricot into which buds from a rosetted peach were inserted June 19, 1920. Note the rosetted shoots produced by the peach buds at *b*, and the apparently healthy growth of the apricot.





# **PLATE 6**

**A.—A healthy plum tree showing the vigorous growth made by August, 1920.**

**B.—The Maynard plum which developed rosette in the spring of 1920. Note the stunted growth and rosetted leaves which developed by August, 1920.**

**C.—The peach seedling into which rosetted Maynard plum buds were inserted June 18, 1920. Note the rosetted plum shoot which developed from one of the buds, and the rosetted peach shoots which developed as a result of inoculation with infected plum buds.**

PLATE 7

A.—The peach tree into which healthy plum buds were inserted June 18, 1920, to serve as a control. Note the healthy growth of the peach stock, and of one plum bud (indicated by the arrow).

B.—The Marianna plum tree into which buds from a rosetted peach seedling were inserted June 18, 1920. Note the rosetted shoot produced by one of the peach buds.

C.—The Marianna plum tree, shown in B, after another season's growth. Note the vigorous, healthy growth of the Marianna stock in contrast to the stunted growth of the peach shoot at *a*, with small rosettes at the tips of the branches.





#### PLATE 8

A.—The Marianna plum tree into which buds from the rosetted Maynard plum were inserted June 18, 1920. Note the vigorous growth of the Marianna branches in contrast to the rosetted shoot, at the right, produced by one Maynard bud after three months' growth.

B.—The Marianna plum stock (same as in A but from the opposite side of the tree), showing the healthy growth of the two Mayflower peach shoots at *a*, in contrast to the stunted, rosetted Maynard plum shoot at *b*.



**PLATE 9**

**A.**—The Mazzard cherry stock into which buds from a rosetted peach seedling were inserted June 19, 1920. Note the stunted growth and rosettes of leaves.

**B.**—The Mazzard cherry stock of the same age as A, into which healthy Elberta buds were inserted as a control.





#### PLATE 10

A.—Bitter almond seedlings growing in the nursery. Note the stunted growth of the tree to the left with a rosetted plum shoot near the base, on May 24, 1922. This tree was inoculated May 23, 1921, with infected wild Chickasaw plum buds. The uninoculated seedlings to the right are healthy, and growing vigorously.

B.—A seedling peach tree into which a bud from a rosetted Bitter almond was inserted February 2, 1922. Note the much branched and tufted growth of the almond shoot which developed from the diseased bud. All the peach shoots which have developed below the point of inoculation have upward rolled leaves, and the lateral and terminal buds have produced small rosettes of yellowish green leaves.



# TOXICITY AND ANTAGONISM OF VARIOUS ALKALI SALTS IN THE SOIL<sup>1</sup>

By F. S. HARRIS, *formerly Director*, M. D. THOMAS, *Associate in Agronomy*, and D. W. PITTMAN, *Instructor in Agronomy*, Utah Agricultural Experiment Station<sup>2</sup>

In the studies of soil alkali which have been carried out at the Utah Station (4, 5)<sup>3</sup> during the past 10 years, a large number of the factors which influence the toxicity of most of the commonly occurring alkali salts have been correlated. It has been frequently noticed, however, that the toxicity of a mixture of salts in a soil seems to be the sum of the separate toxicities of the constituents of the mixture, and since these observations are at variance with the marked antagonistic action of the same salts in solution cultures a more detailed study of this question has been undertaken.

The experiments described in this paper were planned to show the influence on plant growth of adding other salts, as well as acids and manure, to a soil already impregnated with sodium carbonate. The possibility of finding a marked antagonism between some of these added substances and "black alkali" was an incentive to make the scope of the investigation as broad as possible. Since the results from this point of view, however, are largely negative the data are presented as a contribution to the literature on the toxicity of mixtures of alkali salts. Some preliminary work has also been done on soils impregnated with sodium chlorid and sodium nitrate instead of sodium carbonate.

## REVIEW OF THE LITERATURE

Experiments conducted by Kearney and Cameron (6) were among the first to show the ameliorating effect of adding a second alkali salt to a solution which was already toxic to plants. In their work the plants were germinated while not in contact with the alkali salts, and the roots were then held in the solution for 24 hours. The toxicity of the solution, was determined by slight injury to the root tips. The toxic concentrations were not proportional to the concentrations which absolutely prevented growth of the plants, and the toxicity of the salts exchanged places somewhat when the absolute rather than the minimum check in growth was considered. Very small quantities of salts caused slight injury to the roots. The addition of a second cation to a toxic solution was found to reduce the toxicity of the solution more than the addition of a second anion. Sodium in most combinations greatly weakened the toxic action of magnesium. Calcium, especially in the form of the sulphate, markedly counteracted the injurious effect of either sodium or magnesium ions. Calcium sulphate was much more beneficial to the sulphates of sodium and magnesium than to their chlorids. A very

<sup>1</sup> Accepted for publication July 11, 1922.

<sup>2</sup> The authors wish to express their appreciation to Mr. N. I. Butt for his help in calculating and tabulating the data presented in this paper.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 317-318

effective neutralization of sodium carbonate injury was found when calcium chlorid was added to the solution.

The work of Miyake(11) with rice in cultures of magnesium sulphate, magnesium chlorid, calcium chlorid, sodium sulphate, and sodium chlorid showed that while *N/10* solutions of the individual salts were toxic, when two of these solutions were mixed in certain proportions the toxic effect was more or less neutralized. The greatest neutralization of toxicity was observed when calcium chlorid was added to magnesium sulphate or chlorid. Some antagonism was also noticed between chlorids and sulphates of sodium and magnesium, as well as between potassium chlorid and magnesium or calcium chlorid. In a study of the antagonism between sodium and potassium, the salts—sodium nitrate, potassium chlorid, potassium nitrate, sodium chlorid, potassium sulphate, and sodium sulphate—were used. The antagonistic action of the cations on each other was much greater than that of the anions. The greatest antagonism between sodium and potassium usually occurred when one part of one *N/10* solution was mixed with four parts of another.

Osterhout(12) found that antagonism between sodium chlorid and potassium chlorid was greater when one of the salts predominated in the solution than when both were present in nearly equal quantities. Small quantities of ammonium chlorid, magnesium chlorid, or calcium chlorid reduced the toxicity of either potassium chlorid or sodium chlorid, but with calcium chlorid even larger quantities were beneficial.

Kearney and Harter(7) tested the tolerance of eight different kinds of plants to solutions of sodium and magnesium salts and found that calcium sulphate greatly diminished the toxicity of the salts, especially magnesium. Calcium sulphate changed the order of toxicity of the solutions.

Hansteen(3), working with wheat seedlings, has found that calcium compounds exert a beneficial effect on the toxicity of solutions of alkali salts.

A comparison of soils and solutions by Harris(4) showed the antagonism between the common alkali salts to be more pronounced in solution cultures of wheat seedlings than in loam soils. In fact the only consistent case of antagonism observed in soil seems to have been with the nitrates of potassium, sodium, and magnesium at 4,000 parts per million (4, p. 46).

Some of the most positive antagonistic results in soils have been secured by measuring the activity of soil bacteria. By this means it has been shown by different experimenters that there is antagonism between anions as well as between cations of the salts common in alkali soils. This work has been so well summarized in a paper by Greaves(2) that the reader is referred to this publication for a review of this phase of the subject.

Experimenting with barley growing on a clay-adobe soil, Lipman and Gericke(8) found antagonism between sodium chlorid and sodium sulphate and between sodium chlorid and sodium carbonate in the second crop, though there was no antagonism shown between these salts in the crop grown soon after the salts had been added. A slight antagonism was noticed between sodium carbonate and sodium sulphate in the first crop. A marked antagonism between sodium sulphate and calcium sulphate was apparent in both the first and second crops.

To discover the possibilities of applying the principles of antagonism to the correction of alkali as found in field soils, Lipman and Sharp<sup>(10)</sup> secured natural alkali soil containing 6,400 parts per million of water-soluble salts of which 4,590 parts per million were sodium chlorid, 980 parts per million sodium sulphate, and 830 parts per million sodium carbonate. Adding 119 parts per million of sulphuric acid to this soil was found to be especially beneficial to the growth of barley; and up to 451 parts per million, the highest quantity tried, this acid was helpful. Calcium sulphate at the rate of 670 parts per million, ferrous sulphate at 324 parts per million, and manure at 3,240 parts per million all materially improved the crop-producing power of the soil. Copper and sodium sulphates at the rate of 65 and 130 parts per million, respectively, were harmful to the crop.

Lipman and Gericke<sup>(9)</sup>, growing barley on a clay-adobe soil, found that copper and zinc reduced the toxicity of sodium chlorid, sodium sulphate, and sodium carbonate. Marked antagonism was also noticed between these salts, especially between copper sulphate and sodium chlorid when applied to a sand soil.

Caldwell<sup>(1)</sup> found no antagonism between sodium chlorid and any one of the chlorids of calcium, magnesium, potassium, copper, or ammonium, with the possible exception of ammonium and magnesium in certain proportions, when he grew corn in quartz sand. Potassium and sodium were always more toxic together than when only one was present at a given strength. Adding either calcium chlorid or copper chlorid to sodium chlorid appeared to ameliorate the injurious effect of the latter by diluting the solution rather than by counteracting the harmful effects of the sodium salt.

## EXPERIMENTAL WORK

### METHOD

The experiments here reported were conducted in glass tumblers containing the equivalent of 200 gm. of dry soil and the optimum amount of moisture. Wheat was the crop grown. The requisite quantity of sodium carbonate in 10 per cent solution was added to five 7-kilo portions of air-dry soil, and each portion was mixed separately by forcing it through a fine sieve twice. They were then placed together in a large can and shaken thoroughly at intervals for several days before being used. This method of mixing gave a very satisfactory distribution of the carbonate, as was shown by analyses of a large number of samples taken from different parts of the can. To the soil for each tumbler the other materials were then added, in solution whenever possible, together with the necessary water, and the soil was thoroughly mixed on a piece of oilcloth. Ten kernels of wheat were planted in each vessel. The surface of the soil was covered with a thin layer of a mixture of 50 per cent paraffin and 50 per cent petrolatum to prevent surface evaporation. A short glass tube extending to the center of the soil mass from the surface permitted the addition of water as it was needed to keep the weight of the tumbler constant. The mulch was very effective, and it was therefore possible to avoid altering the uniformity of the distribution of the soluble material through irrigation—at least until the plants were fairly large—as the weight of the tumbler did not change appreciably



at first. The plants which came up were counted daily and every four or five days their height was recorded also. At the end of 21 days they were measured for the last time, cut off at the surface of the ground, and dried to constant weight at  $100^{\circ}$  F. Data were thus secured on the days to germinate, the average height, and the dry weight of the plants. Most of the discussion in this paper, however, will be based on the dry weight which is perhaps the most reliable index of growth.

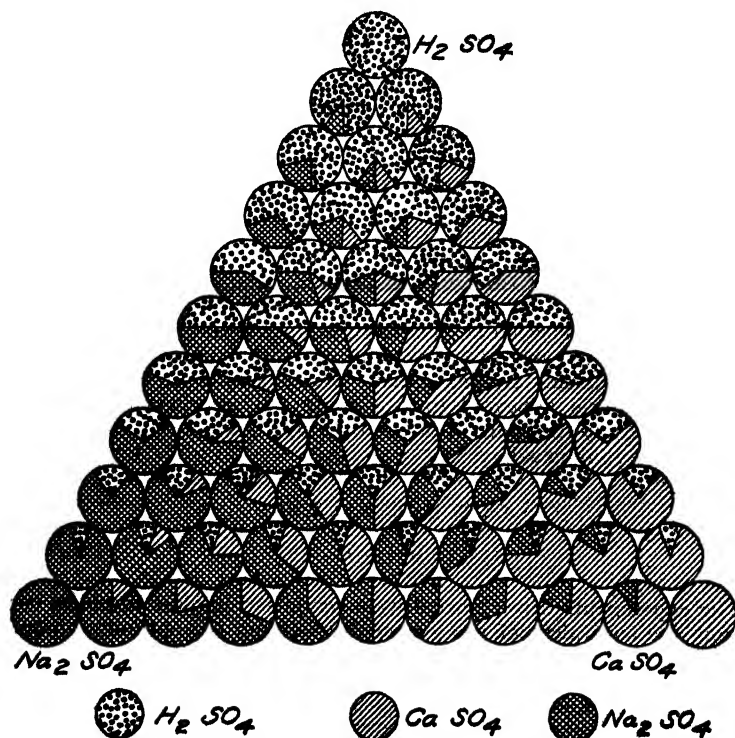


FIG. 1.—Diagram showing the arrangement of the tumblers in the preliminary experiments. A uniform concentration of sodium carbonate was present in all the tumblers, and an equal total quantity of the sulphates was added to each in the relative proportion indicated by the shading.

#### PRELIMINARY EXPERIMENTS

During the summer of 1919 some preliminary work was done on this problem. The technic of the experiment was essentially the same as that described above, except that no mulch was used and the preliminary mixing of the carbonate with the soil was not quite so thoroughly done. The tumblers were arranged in a triangular formation consisting of 66 glasses. All contained a single concentration of sodium carbonate together with sodium sulphate, calcium sulphate, and sulphuric acid in the different concentrations represented in figure 1. The total concentration in parts per million of the added sulphates was always the

same as that of the carbonate, but there were 10 different quantities of each individual sulphate in the triangular grouping. In addition, there were 12 extra tumblers containing the four added substances alone, at their maximum concentrations. A "trial" consisted of 10 of these treatments in which the quantity of carbonate and sulphates added ranged from 1,000 to 10,000 parts per million. Three trials were first carried out with these materials in sand, Greenville loam, and West Logan clay loam. Then two more trials were made with Greenville loam and the same sulphates but with the substitution of sodium chlorid in one case and sodium nitrate in the other in place of sodium carbonate. In the last two trials the concentrations of the salts and also the sulphates ranged from 500 to 5,000 parts per million.

Mechanical and partial chemical analyses of the soils used in these experiments have already been published by one of the writers (13). All the soils contained a large quantity of calcium carbonate. The sand was coarse and low in organic matter. The two heavier soils were nearly identical in texture, but the Greenville soil contained a little more organic matter.

A critical study of the results of this preliminary test has shown that the experimental error in the individual tumblers may frequently be large enough to destroy the regularity of the variations due to the gradual interchanging of the three sulphates so that a presentation of the data in full detail would be confusing. The triangular formation has therefore been divided into seven regions, namely, the center, the three corners, and the middles of the three sides. The value for each region has been found by averaging three to six tumblers. It is fully realized that no two tumblers had exactly the same "alkali" treatment, and, accordingly, this mode of presentation may not seem strictly justifiable, but since it has been observed that the average for three glasses in a given area is usually rather close to the average of six or more tumblers at that place, it is felt that this objection is of minor importance. The results are reported on the basis of the average dry weight per plant. The use of this basis neglects the fact that the percentage of germination in the higher concentrations of alkali is very much reduced and therefore the curves do not fall as rapidly as they would on the basis of total dry-weight production. The results seem quite comparable, however, and, as the relations in the higher concentrations are thus more clearly brought out, it has seemed best to adopt this basis. The data are given graphically in figures 2 to 6, each of which consists of four charts. Chart A shows the results of the sulphuric acid treatment with and without sodium carbonate, while B and C give the corresponding data for calcium sulphate and sodium sulphate, respectively. Finally, the two and three component mixtures added to the carbonate soil are shown in Chart D. The data for the untreated carbonate soil is reproduced in all the charts as a heavy unbroken line.

The results of the experiment with sand are given in figure 2. The beneficial action of calcium sulphate (fig. 2, B) and sulphuric acid (fig. 2, A) on the carbonate soil is forcibly shown. The acid is somewhat more efficacious than the calcium sulphate, as would be expected from the fact that the gypsum is too insoluble to be added in solution and hence would react more slowly. Since the sand contained a large quantity of calcium carbonate, some calcium sulphate was doubtless formed on the addition of the acid, and it therefore seems likely that both correc-

tives functioned in the same way. Sodium sulphate showed a very slight ameliorating action at 1,000 parts per million, at which concentration it appears to be decidedly stimulating to plant growth when added alone, but at higher concentrations it increased the toxicity of the carbonate (fig. 5, C). The mixtures of equal parts of two or three sulphates were all somewhat beneficial, but the presence of sodium sulphate noticeably reduced the corrective power of the mixture in proportion to its concentration (fig. 5, D). All the observations that have been made with the sandy soil can be readily explained by the simple ionic reactions between the added substances, and, accordingly, this case is not essentially different from a solution culture.

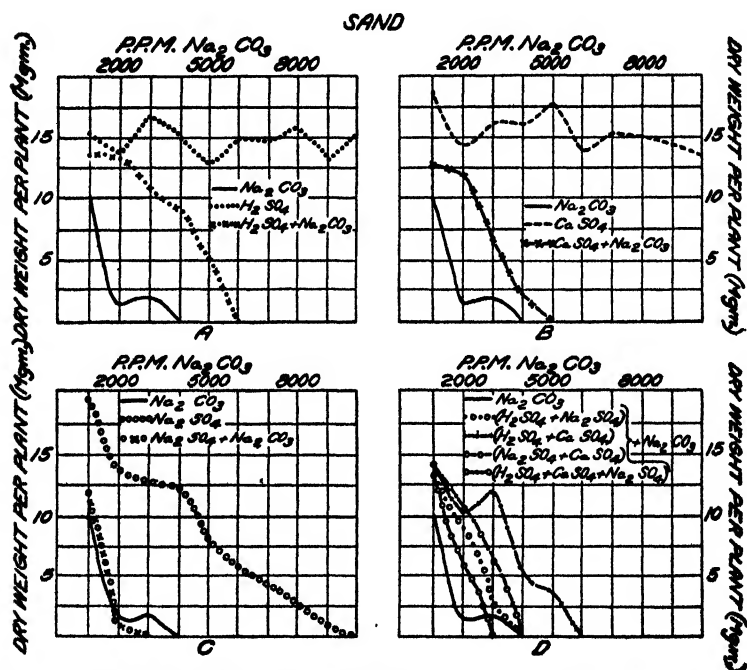


FIG. 5.—Diagram showing the effect on the growth of wheat plants of adding sulphates to sand impregnated with an equal total quantity of sodium carbonate.

In figures 3 and 4 are shown the results obtained when the previously mentioned materials were added to Greenville loam and West Logan clay loam, respectively. Both soils behave similarly and may be conveniently discussed together. As in the case of sand, neither the sulphuric acid nor the calcium sulphate is toxic at any of the concentrations used. The sodium sulphate begins to reduce the growth of the plants appreciably at about 5,000 parts per million, and the mixtures of this salt with sodium carbonate show decided additive toxicity except possibly at the lowest concentrations. For example, the dry weight per plant in Greenville soil is reduced to one-half normal (6.7 mgm.)

by sodium carbonate alone at 7,500 parts per million, by sodium sulphate at 9,500 parts per million, and by a mixture of the two containing 5,000 parts per million each (fig. 3, C). Considering the fact that there is some chemical removal of these salts from the soil solution by the soil material (13, p. 431) so that their concentration is really lower than the amount added indicates, these toxicity relations appear to be very nearly additive.

All the other mixtures follow the untreated sodium carbonate curve much more closely, indicating that they have very little, if any, influence on the toxicity of this salt, though the presence of sodium sulphate in

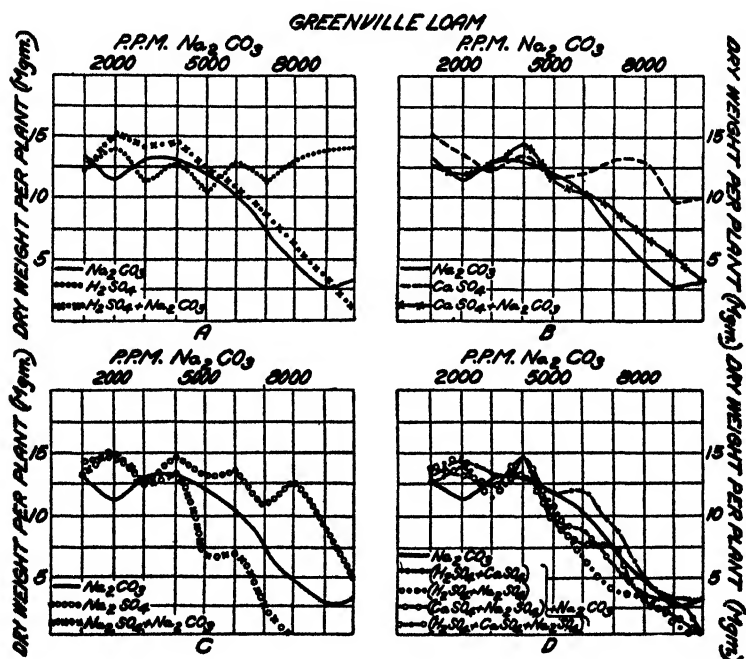


FIG. 3.—Diagram showing the effect on the growth of wheat plants of adding sulphates to Greenville loam already impregnated with an equal total quantity of sodium carbonate.

them is nearly always made manifest by the somewhat lower position of the curve. It is interesting to note that the calcium sulphate when mixed with sodium carbonate does not react in the same way in both soils. In the Greenville soil it shows no harmful effects at all in conjunction with the carbonate, whereas in the West Logan soil it seems to lower the yield slightly. There is certainly no striking evidence of antagonism between any of these substances in either of the two heavier soils.

Figure 5 represents the data for the dry weight per plant obtained by treating the Greenville soil, already impregnated with sodium chlorid, with sulphuric acid, calcium sulphate, and sodium sulphate. The con-

centrations of all these substances ranged from 500 to 5,000 parts per million. The general relations are nearly the same as with the carbonate shown in figures 3 and 4. The simple mixture of sodium chlorid and sodium sulphate shows additive toxicity, while the other mixtures add a much smaller amount or nothing at all to the harmful effects of the chlorid. The three and four component mixtures follow the simpler chlorid curve closely (fig. 5, D). No antagonism for sodium chlorid is evident.

The arrangement of the experiment represented in figure 6 differs from that of figure 5 only in having sodium nitrate substituted for

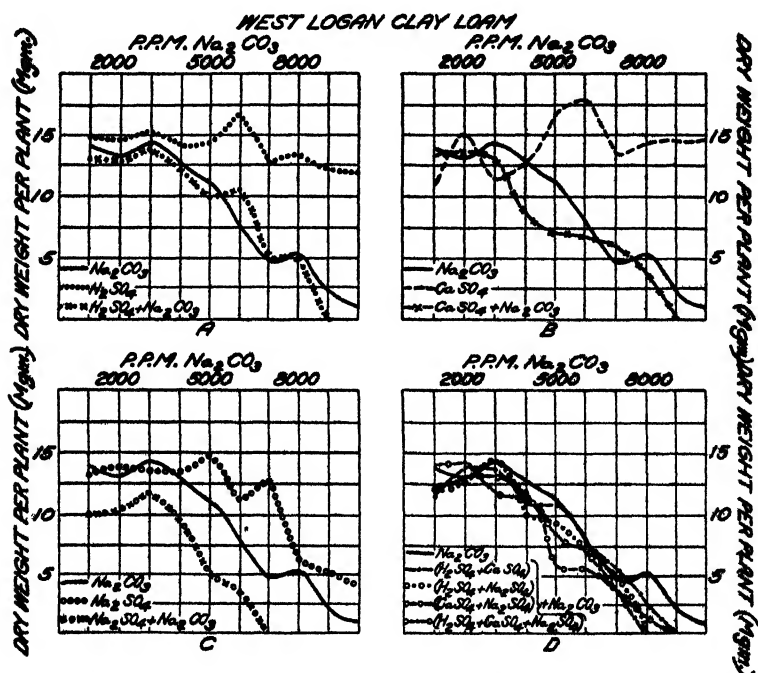


FIG. 4.—Diagram showing the effect on the growth of wheat plants of adding sulphates to West Logan clay loam already impregnated with an equal total quantity of sodium carbonate.

sodium chlorid. The relative shapes and positions of all the curves are so exactly analogous to those in figure 5 that the remarks and conclusions given above apply in this case also.

#### LATER WORK

With the experience gained in the experiments already described, the investigation of this problem was continued in the summer of 1921. It was decided to concentrate effort on one soil, containing one salt as a base, and to vary the subsequent treatment as much as possible. Accordingly, six different trials were carried out with Greenville loam

as the soil and sodium carbonate as the common salt in all the mixtures. In each trial three different substances were added, so that the effects of 15 materials in conjunction with sodium carbonate have been studied.

The triangular arrangement of the experiment was adhered to, but the number of individual treatments was reduced from 66 to 15, as shown in figure 7. In addition there were included in each triangular arrangement five control treatments in which each of the four individual materials and also distilled water were added to the soil alone. The whole experiment was done in duplicate. The same sodium carbonate treatment was present in five of these triangular groupings in which the concentrations

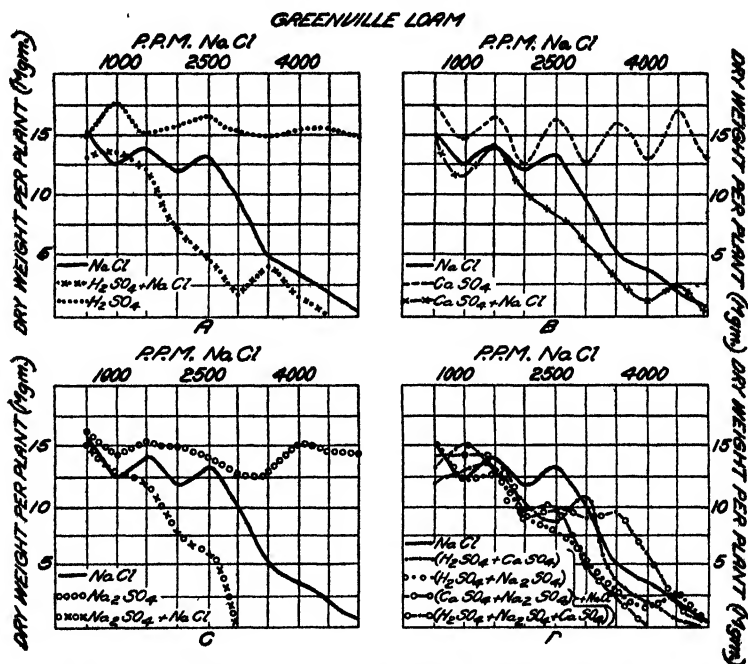


FIG. 5.—Diagram showing the effect on the growth of wheat plants of adding sulphates to Greenville loam already impregnated with an equal total quantity of sodium chloride.

of the other added substances, when they were toxic in themselves, were made to range as nearly as possible from stimulating amounts to decidedly toxic quantities. Four concentrations of sodium carbonate ranging from 1,000 to 7,500 parts per million made up a trial, which thus consisted of 20 units containing 40 tumblers each.

In figures 8 to 13 the results of six trials are presented graphically on the basis of the dry weight per plant. The straight dotted line across all the charts gives the yield secured from the untreated soil, as derived from 40 tumblers. The heavy unbroken line shows the yield from the soil treated with sodium carbonate alone and is the average of 10 tumblers.

It will be noticed that every concentration of sodium carbonate is represented in the figure by three charts containing six curves in each. On

the left is shown the results of mixing the three added substances at their five maximum concentrations with the untreated soil and also with the carbonate soil (fig. 7, tumblers 1, 11, and 15). The center chart represents tumblers 2, 3, 7, 10, 12, and 14 (fig. 7). These contain sodium carbonate and a binary mixture with one of the added substances distinctly predominating. On the right are given the results for tumblers 4, 5, 6, 8, 9, and 13 (fig. 7). These contain the carbonate and both binary and ternary mixtures in which the concentrations are more nearly equal. It should be emphasized that the fractions occurring in the legends to

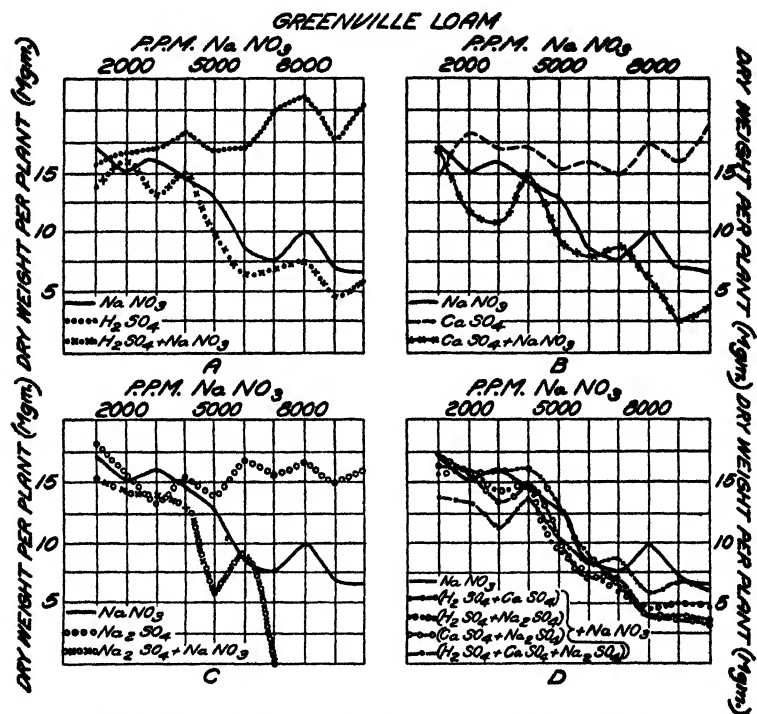


FIG. 6.—Diagram showing the effect on the growth of wheat plants of adding sulphates to Greenville loam already impregnated with an equal total quantity of sodium nitrate.

describe mixtures refer to a proportionate part of the maximum concentration of the unmixed substance and not to the total amount of the mixture. For example, in figure 8, in the second triangle, the maximum quantities of sulphuric acid, potassium sulphate, and sodium sulphate are 4,000, 3,000, and 3,000 parts per million, respectively. Then the mixture one-fourth sulphuric acid, one-half potassium sulphate, and one-fourth sodium sulphate in this grouping contains 1,000 parts per million sulphuric acid, 1,500 parts per million potassium sulphate, and 750 parts per million sulphate, or a total of 3,250 parts per million, and it is this total which is plotted in the curves for this mixture.

SULPHURIC ACID, POTASSIUM SULPHATE, AND SODIUM SULPHATE

The concentrations used in this trial (fig. 8) are as follows:

	Parts per million at concentration—				
	1	2	3	4	5
H <sub>2</sub> SO <sub>4</sub> .....	2,000	4,000	6,000	8,000	10,000
K <sub>2</sub> SO <sub>4</sub> .....	1,000	3,000	5,000	8,000	10,000
Na <sub>2</sub> SO <sub>4</sub> .....	1,000	3,000	5,000	8,000	10,000
Na <sub>2</sub> CO <sub>3</sub> .....	1,000	2,000	4,000	6,000	.....

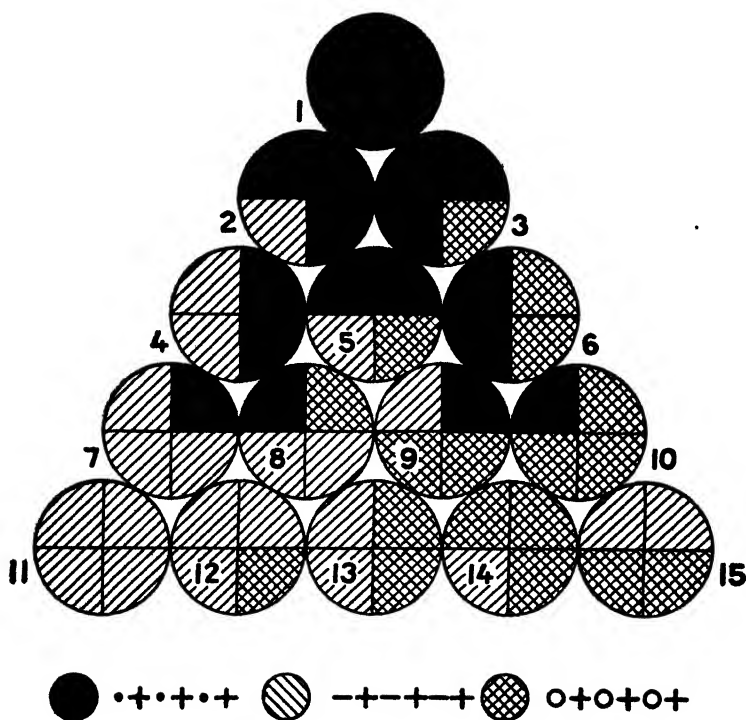


Fig. 7.—Diagram showing the arrangement of the tumblers in the final experiments. A uniform concentration of sodium carbonate was present in all the tumblers and the treatment was added as indicated by the shading. The maximum concentrations used are given in the text.

When added to the untreated soil, the potassium sulphate is only slightly toxic at 8,000 and 10,000 parts per million; the sodium sulphate is very detrimental to plant growth at 8,000 parts per million, and the acid is not harmful at all at 10,000 parts per million. This is indicated by the left section of figure 8, which also shows that the presence of sodium carbonate in the soil lowers the positions of these curves in proportion to its amount. The divergence of these pairs of curves due to



sodium carbonate also increases somewhat as the concentration of the sulphates increase. It is curious that this divergence should be appreciable when the quantity of carbonate present is not sufficient to show

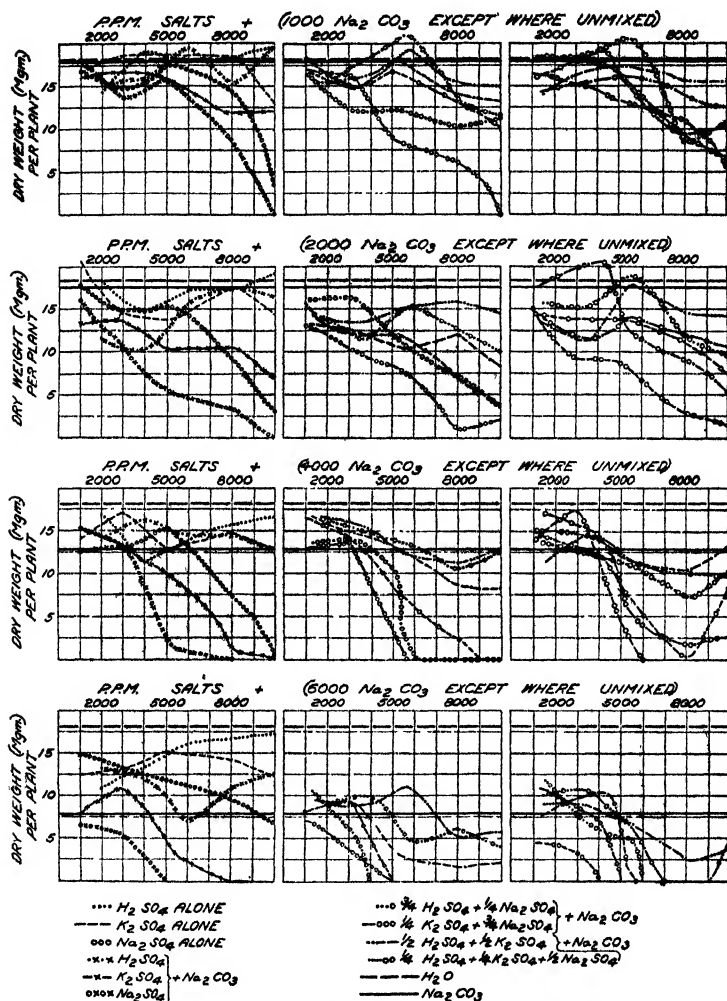


FIG. 8.—Diagram showing the effect on the growth of wheat plants of adding sulphates in various proportions to Greenville loam already impregnated with sodium carbonate. The maximum concentrations of the individual sulphates are given in the text.

any toxicity, and as this condition does not obtain in the other trials it may be due to experimental error in this case. The data are not exact enough to permit quantitative relations to be drawn with certainty, but they seem to indicate that when 5,000 parts per million or more of

the sulphates are present the divergence of the curves is roughly equal to the toxicity of the carbonate.

The values for the binary and ternary mixtures given in the middle and right sections of figure 8 arrange themselves in fairly regular order

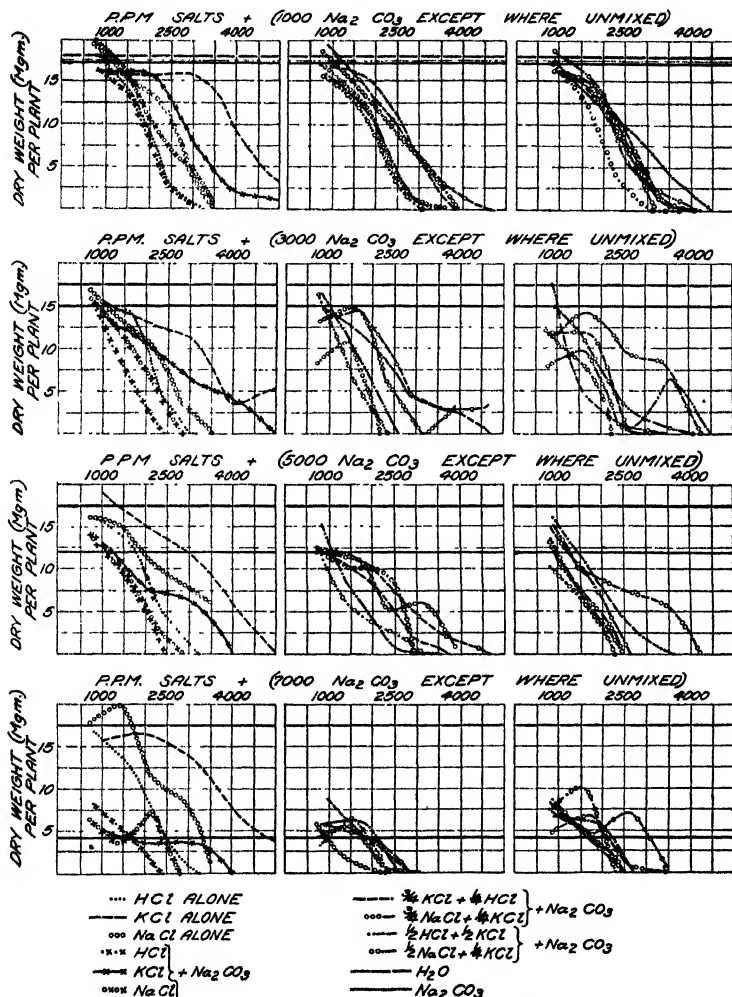


FIG. 9.—Diagram showing the effect on the growth of wheat plants of adding chlorides in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual chlorides are given in the text.

in accordance with the separate toxicities of the components, the individuality of these materials being essentially maintained even in the most complicated mixtures.

It should be particularly noted that the addition of nontoxic amounts of sulphates (up to 3,000 to 5,000 parts per million) to the soil containing appreciably harmful concentrations of carbonate almost invariably causes the growth of the plants to be somewhat better than with the carbonate alone. Three exceptions to this rule appear when sodium sulphate alone and also two mixtures of this salt with potassium sulphate are added to the most toxic black alkali soil. A distinct corrective action or antagonism for sodium carbonate is thus indicated, since no marked stimulation or corrective action is apparent when these substances are added to the untreated soil or to the practically nontoxic carbonate soils.

#### HYDROCHLORIC ACID, POTASSIUM CHLORID, AND SODIUM CHLORID

The concentrations used in this trial (fig. 9) are as follows:

	Parts per million at concentration—				
	1	2	3	4	5
HCl.....	800	1,600	2,400	3,200	4,000
KCl.....	1,000	2,000	3,000	4,000	5,000
NaCl.....	700	1,400	2,100	2,800	3,500
Na <sub>2</sub> CO <sub>3</sub> .....	1,000	3,000	5,000	7,000	.....

The order of increasing toxicity of these substances is potassium chlorid, sodium chlorid, and hydrochloric acid. This order is maintained quite consistently throughout all the mixtures in the trial. The acid probably exerts its harmful properties through being changed to calcium chlorid. This salt is nearly as toxic as sodium chlorid, weight for weight, and the deficiency is more than made up by the fact that two parts of acid produce three parts of calcium chlorid.

It is to be noted again that sodium carbonate at 3,000, 5,000, and 7,000 parts per million lowers the yield of the single chlorid treatments by its own toxicity. A slight correction of the harmful effects of both 5,000 and 7,000 parts per million of the carbonate has been brought about by the lowest concentrations of the chlorids; but this was very slight indeed in the former case, due to the fact that the sodium carbonate was less harmful than usual in this instance.

#### NITRIC ACID, POTASSIUM NITRATE, AND SODIUM NITRATE

This treatment (fig. 10) contains the following concentrations:

	Parts per million at concentration—				
	1	2	3	4	5
HNO <sub>3</sub> .....	1,000	2,000	3,000	4,000	5,000
KNO <sub>3</sub> .....	1,000	2,000	3,000	4,000	5,000
NaNO <sub>3</sub> .....	1,000	2,000	3,000	4,000	5,000
Na <sub>2</sub> CO <sub>3</sub> .....	1,000	3,000	5,000	7,000	.....

The nitric acid is the most toxic material in this trial, as was the hydrochloric acid in figure 9, and the potassium salt is the least harmful. The general similarity of the nitrate and chlorid curves is evident, and

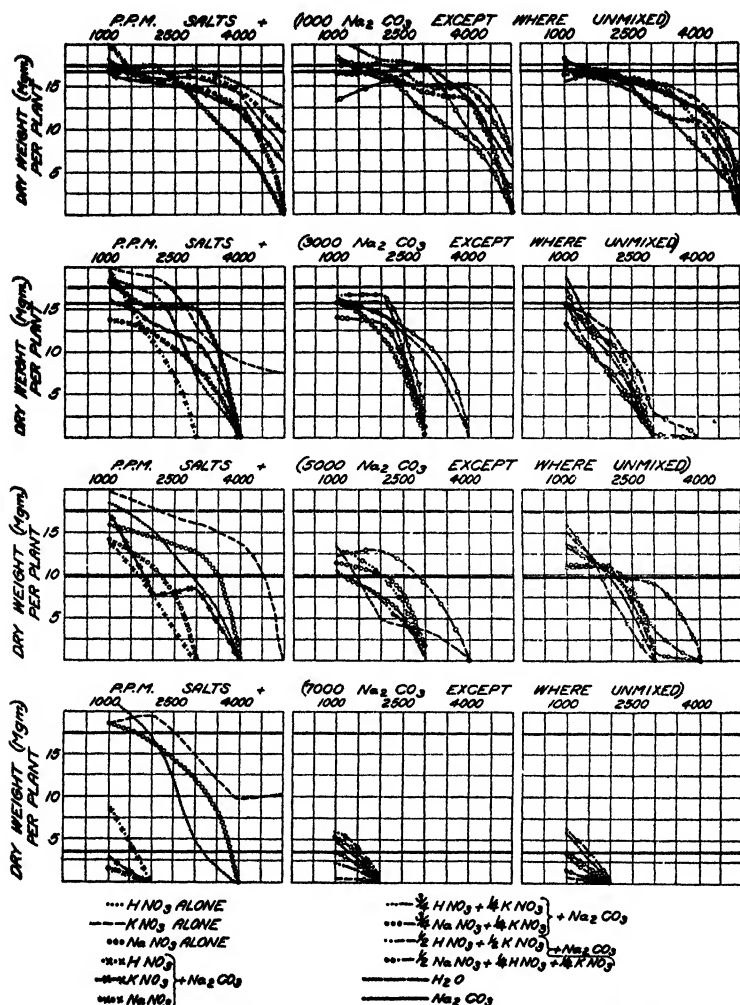


FIG. 10.—Diagram showing the effect on the growth of wheat plants of adding nitrates in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual nitrates are given in the text

the same conclusions may be drawn concerning the additive toxicity of the carbonate and the corrective action of small quantities of the nitrates on this salt. In this case, however, the antagonistic action is fairly large in the third carbonate concentration and negligible in the fourth.

A special point of interest in this trial is the fact that both potassium nitrate and nitric acid are distinctly stimulating to plant growth at 1,000 parts per million. It should also be noted that their corrective action at 1,000 parts per million, as shown in the first and second rows of figure 10 as well as in the third row with 5,000 parts per million carbonate, is appreciably greater than in any of the other diagrams where a nontoxic concentration of an inorganic substance is used, calcium sulphate excepted. Accordingly, their beneficial action in this connection may be due, at least in part, to an increasing of the vigor of the plant growth rather than to the specific reduction of the toxicity of the carbonate which seems to take place in cases of antagonism in solution cultures. Nitric acid and potassium nitrate give promise of real utility in correcting small and moderate toxicities of sodium carbonate in soil.

#### SODIUM CHLORID, SODIUM NITRATE, AND SODIUM SULPHATE

The concentrations of the salts in this trial (fig. 11) are as follows:

	Parts per million at concentration—				
	1	2	3	4	5
NaCl.....	700	1,400	2,100	2,800	3,500
NaNO <sub>3</sub> .....	1,000	2,000	3,000	4,000	5,000
Na <sub>2</sub> SO <sub>4</sub> .....	1,000	3,000	5,000	8,000	10,000
Na <sub>2</sub> CO <sub>3</sub> .....	1,000	3,000	5,000	7,500	.....

The salts are arranged in order of decreasing toxicity in the table. The results of the experiment are in agreement with the general observations which have already been made on the additive toxicity of the carbonate. Nontoxic quantities of all the salts, particularly sodium sulphate, increase the yield in the presence of 5,000 parts per million carbonate, but 7,500 parts per million seems to be so toxic that relief is impossible.

#### DISODIUM PHOSPHATE, SODIUM ARSENITE, AND BORAX

These miscellaneous substances (fig. 12) were added in the following quantities:

	Parts per million at concentration—				
	1	2	3	4	5
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> .....	140	280	420	560	700
Na <sub>3</sub> AsO <sub>3</sub> .....	194	388	582	776	970
Na <sub>2</sub> HPO <sub>4</sub> .....	1,500	3,000	4,500	6,000	7,500
Na <sub>2</sub> CO <sub>3</sub> .....	1,000	3,000	5,000	7,000	.....

The borax and arsenite are 10 times as toxic as the phosphate and they are therefore plotted in figure 12 with one-tenth the phosphate scale. Under these conditions the borax and phosphate curves are nearly coincident, but they are somewhat higher than the arsenite curve.

It is unfortunate that the initial quantity of the latter salt was so large, because no information is afforded concerning the nontoxic concentrations of sodium arsenite, except inferentially from the complex mixtures.

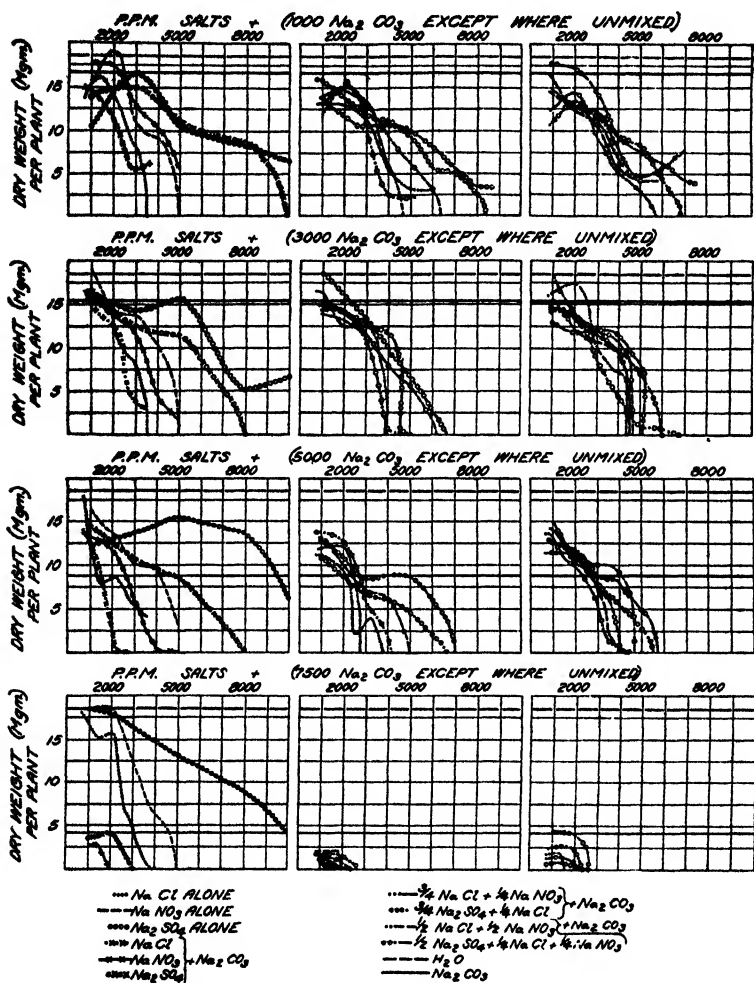


FIG. 11.—Diagram showing the effect on the growth of wheat plants of adding sodium salts to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual salts are given in the text.

The usual regular arrangement of the mixture curves and also the additive toxicity of the carbonate is evident in the diagram. No corrective action on black alkali, however, is shown in this experiment.

The physiological action of the borax and arsenite on the wheat plants was so striking and so characteristic that it should be mentioned here. Borax caused the leaves of the plants to remain closed up around

the stock, which then twisted itself into curious shapes, at the same time exhibiting a distinct chlorotic condition. The intensity of these symptoms varied with the concentration of the borax. They were dis-

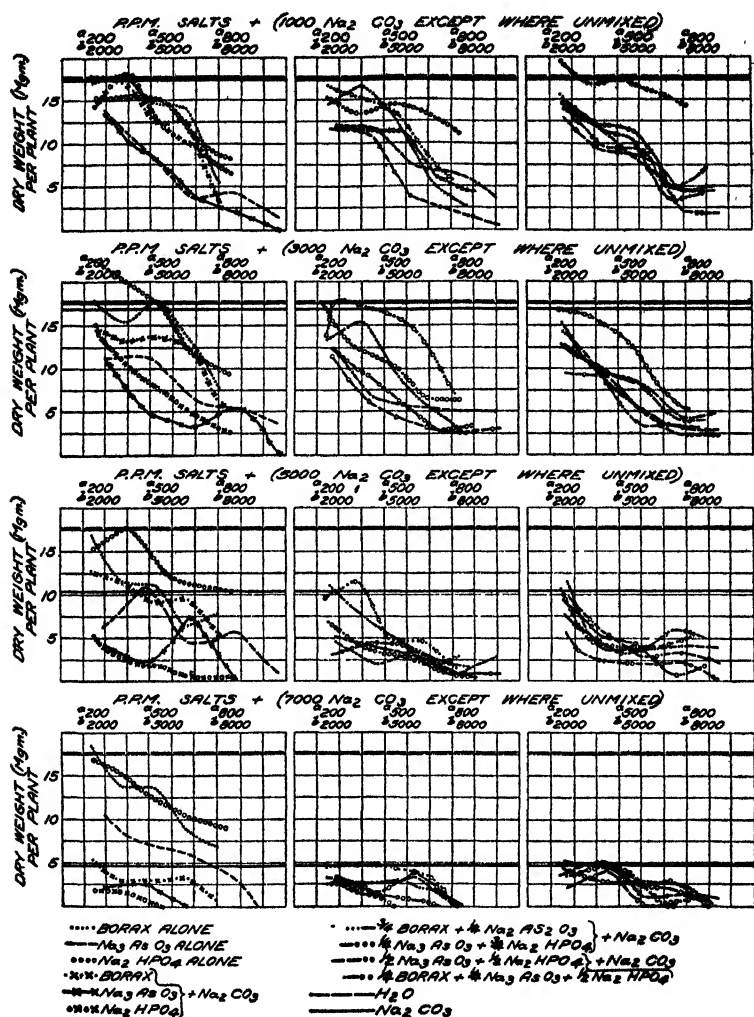


FIG. 12.—Diagram showing the effect on the growth of wheat plants of adding miscellaneous sodium salts in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual salts are given in the text.

\* This reading applies for a sodium arsenite or borax mixture or where used alone.

\* This reading applies for sodium phosphate mixtures or where used alone.

cruible with as little as 70 parts per million, though at the lowest concentrations they tended to disappear as the plant grew. The arsenical poisoning was characterized by a stunted growth, straight, leafless stock, and a dark, unhealthy color.

CALCIUM SULPHATE, SULPHUR, AND MANURE

For the sake of completeness three well-known black alkali amendments were included in the experiment. Since none of them were toxic, the concentrations were made to range beyond the limits set by agricultural practice:

	Parts per million at concentration—				
	1	2	3	4	5
CaSO <sub>4</sub> .....	1, 000	3, 000	6, 000	10, 000	20, 000
Sulphur.....	200	1, 000	3, 000	6, 000	10, 000
Barnyard manure.....	2, 000	5, 000	10, 000	15, 000	20, 000
Na <sub>2</sub> CO <sub>3</sub> .....	1, 000	2, 000	5, 000	7, 000	.....

The materials were mixed with the soil in a dry, powdered condition before the water was added. With the larger quantities of manure the soil was somewhat deficient in moisture because of the larger absorptive power of this treatment for water. The results for manure can not, therefore, be taken as final, since under other moisture conditions a different behavior might be expected.

The experiment (fig. 13) shows that all three added substances are somewhat stimulating alone and that all have the power of counteracting partially the toxicity of sodium carbonate. This corrective action is accomplished in the lower carbonate treatments about equally well by all three materials, but with sodium carbonate at 7,500 parts per million calcium sulphate is the most effective and even seems to be a necessary component of the amending mixture. It should be noted that curves with 7,500 parts per million sodium carbonate represent average values for two entirely independent but closely concordant experiments.

SUMMARY

(1) The toxicity relations for wheat of certain alkali salts alone and in combination with each other have been investigated with special reference to the alleviation of black alkali trouble.

(2) It has been shown that when a given concentration of sodium carbonate in sand is treated with an equal quantity of calcium sulphate or sulphuric acid, an appreciable lowering of the carbonate's toxicity is evident. This relation, however, is not so manifest in heavier soils. Sodium sulphate increases the toxicity of the carbonate under the same circumstances.

(3) Either sodium chlorid or sodium nitrate could be substituted for sodium carbonate in the arrangement described above without changing the results. No antagonism between these salts and the sulphates could be detected in Greenville loam.

(4) During 1921 each one of four different concentrations of sodium carbonate, already incorporated in the Greenville soil, has been treated with five concentrations of the following substances, in groups of three:

- (1) Sulphuric acid, potassium sulphate, and sodium sulphate.
- (2) Hydrochloric acid, potassium chlorid, and sodium chlorid.
- (3) Nitric acid, potassium nitrate, and sodium nitrate.
- (4) Sodium chlorid, sodium sulphate, and sodium nitrate.
- (5) Sodium arsenite, sodium phosphate, and borax.
- (6) Calcium sulphate, sulphur, and barnyard manure.



(5) The toxicities of all the substances in the first five groups are increased by the specific toxicity of sodium carbonate when they are added to soil already impregnated with this salt.

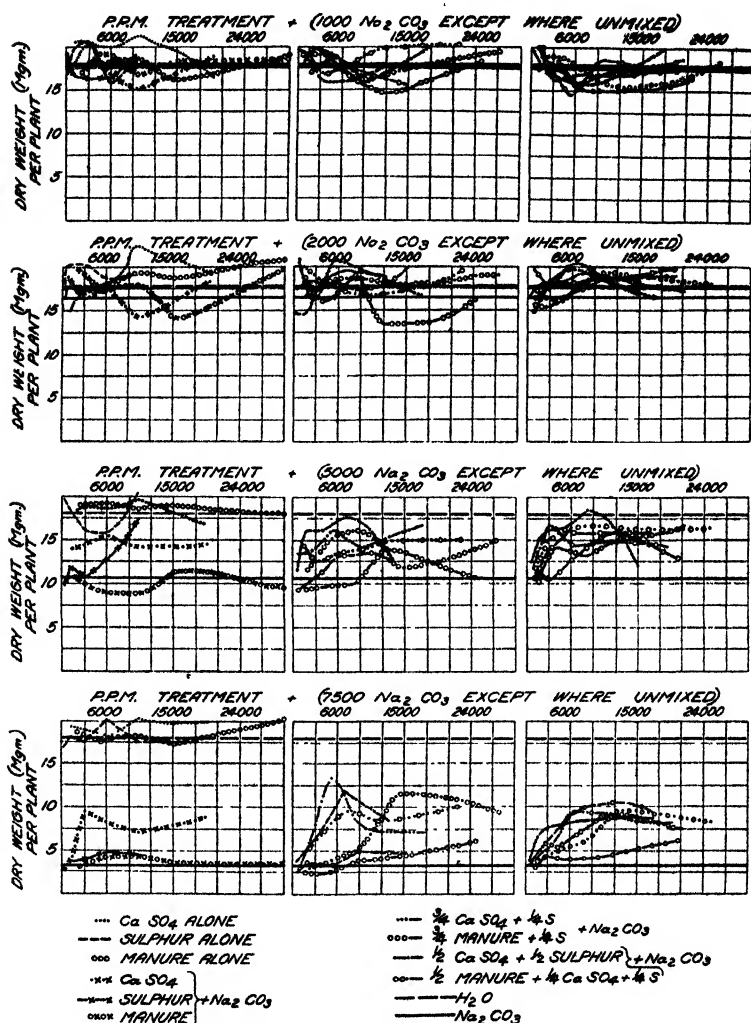


FIG. 13.—Diagram showing the effect on the growth of wheat plants of adding manure, sulphur, and gypsum in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual added substances are given in the text.

(6) Nontoxic quantities of all the substances in the first four groups have the power of correcting, to some extent at least, the harmful effects of moderate, and in some cases of fairly high, concentrations of sodium carbonate. The salts in the fifth group do not exhibit this property at

all. This phenomenon may be due in part at least to specific stimulation of plant growth by these substances rather than to any antagonistic action on the sodium carbonate which would lower the toxicity of that salt as is frequently observed in solution culture.

(7) Potassium nitrate and nitric acid are both distinctly stimulating to plant growth at 1,000 parts per million, and the addition of this quantity of these materials to soil containing 5,000 parts per million sodium carbonate or less was particularly beneficial.

(8) Under the conditions of the experiment, barnyard manure was an effective amendment for soil containing 2,000 parts per million sodium carbonate, but its corrective power for more toxic concentrations of black alkali was much less evident, probably because the optimum moisture content of the soil was not maintained when the larger amounts of manure were added.

(9) Calcium sulphate, alone and in conjunction with sulphur and manure, is the most effective corrective that was tried, particularly on the more toxic black alkali soils.

(10) The curious physiological effects of borax and sodium arsenite on the wheat plants have been noted.

(11) While the data herein presented throw some light on the antagonistic action of various alkali salts, it is evident that the problems of coping with black alkali is far from being solved.

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# IDENTIFICATION OF CERTAIN SPECIES OF FUSARIUM ISOLATED FROM POTATO TUBERS IN MONTANA<sup>1</sup>

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## INTRODUCTION

The economic importance to this State of Fusarium wilt and various storage and field rots of potatoes has been recognized for several years. Between 1914 and 1918 isolations were made from affected tubers until nearly 100 cultures had been obtained. These isolations have been cultured on artificial media, being transferred about twice a year and kept in a refrigerator at about 10° C.

The purpose of this paper is to record the taxonomic work on these various Fusarium cultures. No especial endeavor was made to connect any of these species with an ascigeral stage, and in all our work no culture showed any form of growth suggesting such a stage.

## SOURCE OF MATERIAL

The tubers from which the isolations were made came from many different localities in the State. In fact, practically all sections except the extreme northwestern are represented. The exact source of each culture will be found in Table I. Of the 97 original isolations only 70 were used.

There is little doubt that dryrot and Fusarium wilt are of large economic importance to all potato growers in the State. In 1917, this Station reported in the plant disease survey a loss of 4 per cent in the potato crop due to wilt and a loss of 3 per cent due to dryrot. The year 1917 is considered a normal one. These figures therefore represent the average yearly loss due to potato diseases caused by species of Fusarium. This loss is fairly evenly distributed over the State.

## LITERATURE REVIEWED

A great deal of work has been done and a number of papers have been written on Fusarium troubles of potatoes, but the viewpoint in nearly all of this work is economic. However, in reporting on the economic phase many of the authors include some taxonomic data, and for this reason the following reviews are given. Occasional comment on individual papers has been made, but this has not been done consistently throughout. For convenience the various papers are taken up in chronological order.

Smith and Swingle (19)<sup>2</sup> found that there was always present in the darkened vascular bundles of tubers affected with wilt, which had not at that time been separated from dryrot, a fungus which on culturing they found to be a species of Fusarium. In order to determine the characters of this fungus they grew it on about 40 different media and under various temperature conditions. The bulletin gives in detail the results of these

<sup>1</sup> Accepted for publication July 24, 1922.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 363-364.

studies. To this organism the authors applied the name *Fusarium oxysporum* Schlechtendal because they felt it "not at all certain" that the various names given to species of *Fusarium* growing on potatoes really stood for distinct forms. They therefore considered them as synonyms and used the earliest available name. This paper was the pioneer for *Fusarium* work in this country, and in addition to the careful work done, mycologists and pathologists are greatly indebted to its authors for the impetus given to the study of potato *Fusaria*, both in this country and abroad.

In 1910 Appel and Wollenweber (2) published a basis for a monograph of the genus *Fusarium* Link. This paper was the first to give a comparatively exhaustive treatment of the species of *Fusarium*. It is divided into two parts. In the first part the following subjects are discussed in detail: Methods, including media, inocula, nutrients, light, temperature, color standards, variation in forms appearing in the cultures, mycelium, etc., lack of distinction between microconidia, and macroconidia and characters which constitute a "normal" culture or "normal" spore. These authors give a description of the genus *Fusarium*, discuss its relationships, and list its synonymy.

The second part relates entirely to taxonomy. Thirteen known species are described with the greatest care and detail, each description being the record of a research problem in itself. This paper is, without doubt, the most fundamental in the literature on *Fusarium*. It is not here reviewed in proportion to its worth, because our experimental work was not directly influenced by it. The paper was published in Germany 10 years ago and did not include many of the common American species. Therefore, it was not well fitted for our identification work. The paper contains a very good bibliography of the *Fusarium* problem.

In 1912 Jamieson and Wollenweber (9) described the symptoms of a disease causing dryrot of potato tubers, first noted on tubers sent from Spokane, Wash., in February, 1910. To the causal organism of this disease Wollenweber gave the name *Fusarium trichothecioides*, placing it in the *Discolor* group. Inoculation experiments proved the pathogenicity of this organism in producing the characteristic dryrot of tubers.

In 1912 Wilcox, Link, and Pool (20) published a research bulletin in which they described a rot which is practically identical with that described by Jamieson and Wollenweber (9), but due to the fact that the two papers were published so close together and that the investigational work was being carried on simultaneously and independently, these authors gave to the casual organism the name *Fusarium tuberivorum* Wilc. and Link, which we know now to be a synonym of *F. trichothecioides* Wollenw. Wilcox, Link, and Pool's paper is of particular value to our work because of its emphasis on taxonomy.

This paper gives the history and distribution of the dryrot of tubers and the economic importance and symptoms of the particular dryrot under discussion. The authors give a résumé of the genus *Fusarium* and allied genera from 1809 to date, concluding with Appel and Wollenweber's description of it.

Details are given concerning the technic for studying the fungus, such as temperature, light, single-spore isolation, and color standard; and a discussion is made of macroscopic characters, mycelium, influence of temperature, influence of humidity, color characteristics, conidiophores, chlamydospores, spore measurements, and spore septations. Considerable space is given to the last-mentioned topic. The importance of

various conditions which might influence the number of septations is discussed, and the results of actual investigations are recorded in detail.

In summarizing the taxonomic portion of the paper the authors endeavor to find a logical place for their species, and after several suggestions and comparisons they leave it unplaced, but give a detailed description of the organism. Some pathological studies are discussed with emphasis on the mode of infection, resistance, and susceptibility of varieties and methods of control.

Wollenweber (21), studying the genus *Fusarium* taxonomically, came to a few definite conclusions: The stroma as a taxonomic character in species determination is unreliable. A pure culture method which gives the normal stages is necessary, and the culture media recommended was steamed stems of trees, shrubs, or herbaceous plants for conidia and chlamydospore production and rice, potato tubers, and other starchy media for secondary characters, such as color, extra large sclerotia, and stromata. He is not sure that *Fusarium* is an obligate conidial stage of an ascomycete. He puts much emphasis on the importance of the proof of pathogenicity of the organisms, and in his discussions of each species he indicates the kind of parasite.

Wollenweber was the first to assemble into sections species of *Fusarium* having related characters. He considered a uniform shape of conidia the most important of the characters on which the division could be based. The sections which he described in this paper have been used in all taxonomic work with this genus since that time. Other sections, of course, have been added, but the original sections have for the most part never needed to be amended.

The 20 species of *Fusarium* described, of which only 3 are new, are grouped under the sections, practically all of which include wound parasites capable of destroying parenchymatous tissue, except the first, which includes vascular parasites only. Wollenweber's sections in their order are: *Elegans*, *Martiella*, *Discolor*, *Gibbosum*, *Roseum*, and *Ventricosum*.

Later Wollenweber (22), in morphological and pathological study of the divisions of fungi having cylindrical and crescent-shaped conidia, states definitely that fungi with cylindrical septate conidia fall outside the genus *Fusarium*, and belong, when the perfect form is known, to the genera *Nectria*, *Hypomyces*, and *Mycosphaerella*, and, when the perfect form is not known, to *Cylindrocarpon* when chlamydospores are absent and to *Ramularia* when they are present.

Lewis (11), working in Maine, carried on comparative studies with some 40 different cultures of *Fusarium* isolated from various hosts, including 7 isolated from potatoes. He noted how the growth of the cultures was effected by different media, different quantities of acid and alkali, and different temperatures. He tested for gas in fermentation tubes, but obtained negative results only. Tests of pathogenicity were carried on with all of the cultures, and considerable cross inoculation work was done. He made no attempt to identify the species, because the published descriptions were so incomplete as to make critical comparisons with them impossible. However, Wollenweber was in the United States at that time, and Lewis sent his cultures to Wollenweber, who identified most of them. His results were added as an appendix to the bulletin.

Four of these species—*Fusarium poae*, *F. helianthi*, *F. conglutinans*, and *F. piri*—do not later appear in the literature as occurring on potato. Sherbakoff (18) says of the first two that they are closely

related to *F. sporotrichioides* n. sp. and belong to the section *Sporotrichiella*; the third species belongs to section *Elegans* and is closely related to *F. orthoceras*; and the fourth may belong to section *Arthrosoriella*. Sherbakoff does not recognize any of these species but disposes of them all in saying:

No technical description, except results of inoculations for potatoes, always negative, and certain characters of color and of colony growth, is given, and thus a proper identification is rendered impracticable.

Harter and Field (7) from the results of their work on the stemrot of the sweet potato concluded, as Appel and Wollenweber (2), that the type of inoculum—mycelium or spores—has a marked influence on the culture. They also proved the pathogenicity of *Fusarium hyperoxysporum* Wr. and *F. batatis* Wr. for the sweet potato and obtained negative results in their inoculation experiments with *F. oxysporum* Schlecht., *F. orthoceras* Ap. and Wr., *F. caudatum* Wr., and *F. radiculicola* Wr. on the same host.

Wollenweber (23) discussing the species of *Fusarium* occurring on sweet potatoes, points out the necessity of agreeing on the criteria by which a "normal" culture may be known, to avoid wide discrepancies in describing what is in reality the same species. He describes 11 species of *Fusarium*, two species of *Hypomyces* and one of *Gibberella* occurring on sweet potatoes. This included all the species of *Fusarium* then known to occur on sweet potatoes. Under each species is given a "diagnosis" or description of the type culture, habitat, and a general discussion of its history and relationships. In the case of new species, of which there are 6, the relationships are taken up with a great deal of care.

A descriptive key to all these species is included based upon the characteristics of pure cultures grown in daylight. Regarding the key the author makes the following comment:

This key might have been based entirely upon the morphological characters and curvature of the conidia but since the color reactions offer a simpler, though less trustworthy means of identification, they have been employed. This key, therefore, should be regarded only as an aid in identification, not as a guide to the morphology, which has been discussed in the diagnosis and illustrated in detail in the illustrations.

Sherbakoff (18) realizing the chaotic condition of the genus *Fusarium*, especially those species occurring on potato, conducted a research problem to discover on what basis the American species as well as those discussed by Wollenweber could best be separated.

In general, Sherbakoff verified the principles and the results of Appel and Wollenweber's (2) work in Germany. He believed, however, disagreeing with Appel and Wollenweber, that there should be a distinction drawn between microconidia and macroconidia, and that the presence or absence of the microconidia may be used in distinguishing species. He also disagrees with Appel and Wollenweber in believing that species can be distinguished—

when grown on almost any medium, including artificial media, provided that the medium is not extremely poor or rich in food materials, and also provided that the moisture supply in the medium is well regulated.

Sherbakoff found no coremia nor typical pionnotal form of fructification as did Appel and Wollenweber.

The author outlined the scope of the work, discussed the source of material and methods of isolation, culture media, effect of light and temperature, variability in the species of *Fusarium*, relative taxonomic

importance of different characters, defined certain forms of fructification, reviewed the genus *Fusarium*, and explained the difficulties of identifying species of *Fusarium* with previously described species because of the scarcity of taxonomic detail in the descriptions. The greater part of the memoir is taken up by the description of sections, genera, species, and varieties. He listed 20 previously described species, including parasitic and nonparasitic forms—all that were known to occur on potatoes—and 41 new species and varieties. These 61 forms he distributed under Wollenweber's eight sections and three additional ones that he himself originated and defined. Three species of *Ramularia*, a genus closely related to *Fusarium* and also occurring on potatoes, are included. There are drawings of practically all species and varieties, showing conidia, conidiophores, and occasionally mycelium. Spore measurements are given for spores grown on various culture media.

Based on the ideas of Appel and Wollenweber, Sherbakoff (18) worked out a dichotomous key for the species of *Fusarium* described, which, while the best yet published, is far from perfect. Imperfections in keys are rather inevitable until methods used in the identification of the species are better standardized. Until standard methods are adopted, the boundaries of species can not be closely enough defined to prevent investigators from introducing numerous varieties, separating one from another on minor characters that are not stable under all conditions.

In the key, Sherbakoff uses septations and shape of conidia most often as differential characters. Presence or absence of microconidia, chlamydospores, sclerotia, sporodochia, type of fructification, and color and type of conidiophores are also used. Difficulty with the key arises most often in the case of varieties. Individual difficulties of this sort will be noted later. Confusion often arises from the misuse of the terms macroconidia and microconidia, but this may be due to typographical errors.

C. W. Carpenter (4), in a paper on tuber-rots caused by species of *Fusarium*, includes a section on taxonomy which gives the description of eight species. Among these one is new, *F. eumartii*, which falls into the section *Marteilla*.

Link (13) shows some very interesting results from physiological studies on *Fusarium oxysporum* Schlecht and *F. trichothecioides* Wr. Comparisons of the two species are given to show temperature relations, growth, habit, and food requirements and pathogenicity to tubers and growing plants.

Pratt (15), in a paper on control of powdery dryrot caused by *Fusarium trichothecioides* Wr., concludes that this species is of the highest economic importance of all of the *Fusarium* species in the irrigated portion of the West, and in another paper (14) notes that *F. radicola* Wr. is rather common in desert soils.

Hawkins (8), in studying the effect of certain species of *Fusarium* on the composition of the potato tuber, found that *Fusarium oxysporum* and *F. radicola* secrete sucrase, maltase, xylase, and diastase. The last-mentioned enzyme is apparently incapable of acting on the ungela-tinized potato starch. The purpose of the study was to find out what constituents of the potato are most easily destroyed by the fungus and what compounds can not be utilized by it either in respiration or in building its own tissues. Their results are not conclusive as to whether kind or quantity of secretion is in the least specific, as only three organisms were used in their experiments, but this article introduces an interesting phase of the *Fusarium* problem.



Pratt (16), in studying the relation between soil fungi and diseases of the Irish potato in southern Idaho, isolated among many other fungi 14 species of *Fusarium*. Five of the strains isolated "apparently differed from all species heretofore described," and Pratt, therefore, named them, giving in this publication the original descriptions, which include habitat, cultural characteristics, spore shape, septations, and size. Septations and size are given of spores grown on various media for different ages.

The new species described are: Section *Gibbosum*, *Fusarium lanceolatum*; Section *Elegans*, *F. elegantum* and *F. Idahoanum*; Section *Discolor*, *F. aridum* and *F. nigrum*.

The other nine species were identified, but in only one case, that where Pratt's culture showed some differences from the "authentic culture" which he used for comparison, are there any taxonomic notes.

Bisby (3) in his studies on *Fusarium* diseases in Minnesota notes that *Fusarium oxysporum* and *F. discolor* var. *sulphureum* are of large economic importance in Minnesota. His results with certain temperatures and media in studying these diseases are of interest to use, but otherwise the bulletin is strictly economic in its outlook.

Edson and Shapovalov (5) made a careful study of the relations of growth of certain species of *Fusarium* to temperature. The species they used in the studies were: *Fusarium discolor*, var. *sulphureum* (Schlect) Ap. and Wollenw., *F. eumartii* Carp., *F. oxysporum* Schlect, *F. radicola* Wollenw., *F. trichothecioides* Wollenw.

Two species of *Verticillium* were also used. For each of these species they made nine plate cultures and incubated them at nine different temperatures from 1° C. up to 40° C. at 5° intervals, taking readings of the size of the colony at the end of each 24 hours. The results, aside from aiding in control determinations, proved to the authors that temperature tests in certain cases may serve as a useful supplementary method for the identification of fungi exhibiting contrasting thermal relations.

#### THE GENUS *FUSARIUM* LINK.

The genus *Fusarium* is classified according to Engler and Prantl *Natürliche Pflanzenfamilien* (6) as belonging to the section *Mucediacae* *Phragmosporae* of the family *Tuberculariaceae* of the order *Hyphomycetes* of that heterogenous class known as the *Fungi Imperfecti*. It is, consequently, a form genus, and already the ascigerous stage of a number of its species has been found. A few of these are *Nectria solani* (Ren. and Bert.), which has been reported as the ascigerous stage of *Fusarium solani*; *Nectria graminicola* B and N., as the ascigerous stage of *F. nivale*; and *Gibberella saubinetii* (Durieu and Mont.) Sacc. to which species *F. tulmorum*, *F. avenaceum*, *F. hordei*, and *F. heterosporum* have all been referred. It seems very probable that more and more species of this genus will be connected with genera of the *Ascomycetes*, though as Wollenweber states (21)—

We are still far from having conclusive proof of the widely recognized theory that *Fusarium* is the obligate conidial stage of *Ascomycetes*.

The genus *Fusarium* was described in 1809 by Link (12), together with the allied genera *Fusidium*, *Fusisporium*, and *Attractium*. Later Link dropped one or the other or combined them in various ways. Schlectendahl, Corda, Fries, and Saccardo worked on this group of organisms and

classified them in various ways, but they all recognized the imperfections of the classification. In their monograph Appel and Wollenweber (2) have established the boundaries of the genus *Fusarium*, using *Atractium* Link, *Fusidium* Link, *Fusisporium* Link, *Selenosporium* Corda, *Fusoma* Corda, and *Pionnotes* Fries, either in toto or in part, as synonyms.

The synonymy of the genus *Fusarium* given by Appel and Wollenweber (2) is quoted below, and a translation is given of their description and notes.

**Synonymy:**

*Atractium* Link pr. p. in Mag. Ges. Nat. Freunde III, S. 10 (1809).

*Fusidium* Link pr. p. in Mag. Ges. Nat. Freunde VII, S. 31 (1816).

*Fusidium* Link pr. p. in Spec. Plant 11, S. 96 (1825).

*Fusisporium* Link in Spec. Plant 1, S. 30 (1824).

*Selenosporium* Corda Icon. I. S. 7 (1837).

*Fusoma* Corda Icon. I. S. 7 (1837).

*Pionnotes* Fries Sum. Veg. Scand., S. 481 (1849) Sacc. Syll. IV, S. 725.

Conidia more or less polar, mostly dorsiventral, seldom distinctly round (radiär), more or less curved; when ripe usually septated; more or less colored when in masses; borne one after another in the same spot, but not connected in chains on the end of simple or branched septate conidiophores which appear spread out between the hyphae or joined as they are in coremia, or grouped together in sporodochia. Conidia spread out in a powdery form between the hyphae or tubercular-like on a limited gelatinous sporodochia, a slimy layer or occasionally as pionnotes without definite boundaries.

Chlamydophores, oval or pear shaped, single or in bunches, in chains or bunched up, remaining joined for some time, terminal or intercalary, not more than one borne in the same place. The chlamydophore is not very different from the conidiophore, and it has no distinctive color. It never gathers in gelatinous layers.

Hyphae septate, variously branched epi- and endo-phytic, occurring sparingly or in great quantity, either isolated or together, curly or thick, partly like coremia, or especially like a stroma to plectychymatic form with definite shape or without definite shape, more often similar to an even growth all over, limited or spread out, often closed up together on the inside, occasionally building up brightly colored mycelium.

Note that it is undecided whether species that do not have septate conidia should be kept separate from the genus or be placed in a subgenus *Fusamen* according to Saccardo (17); but there is no question about those which have a tendency toward septation as *F. orthoceras*. It is also undecided in what order of importance the characters should be taken. The choice is between septations, dorsiventrality, polarity and the curve of the long axis of the conidia. It is very questionable whether *Fusarium* should be placed under *Leptosporium* as in Saccardo, and nothing but the study of the different forms can decide the boundaries of the genus. Concerning the color of the conidia masses it can be said that black does not appear normally, neither does black mycelium. Light orange and ochre colors predominate in the conidia. The mycelium also has yellow, red and blue. The term sclerotium as used in *Fusarium* is disputable. Researches have not shown that the term sclerotia was justifiable for the plectychymatic structures found.

In 1913 Wollenweber (22) excluded from the genus all species having septocylindrical conidia. In bringing this genus to date, therefore, this fact should be incorporated. Sherbakoff (18) describes the genus concisely as follows:

Hyphomycetes, with from hyaline to bright, but never plain gray nor black, conidia and mycelium; conidia sickle-shaped, septate (usually 3 or more septate), apically pointed, mostly pedicellate, not appendiculate, noncatenulate; conidia scattered over substratum, in pseudopionnotes or in sporodochia, the latter without or with from flat to wart-like plectenchymic substratum, and always without any differentiated enclosing or surrounding structures; conidiophores from simple to irregularly verticillate.

## ISOLATION OF CULTURES

The original cultures were all obtained from infected tubers by the following method:

The infected tubers were thoroughly washed, dipped into a 1 to 1,000 solution of mercuric chlorid, and cut open. In order not to contaminate the infected parts of the tuber, the healthy portion was cut almost to the edge of the discolored portion and the tuber then broken open, care being taken that nothing should touch the advancing margin of the fungus. A piece of discolored tissue was taken from the advancing margin and put into a tube of melted agar, which was then poured into a Petri dish and incubated at room temperature. Six to 10 plates were made from a single tuber. The plates were watched carefully to be sure that a pure culture was the result of the isolation. When this was assured a transfer was made to agar slants, and these were kept as stocks. It was surprising how very few contaminations appeared in the plates. Each culture was numbered, and all notes, including source, were kept under this number.

## SINGLE-SPORE ISOLATIONS

In 1916 when detail work with these cultures was anticipated it seemed best to take every precaution to be assured of pure cultures, for a mixed culture of two or more species of *Fusarium* could easily pass unnoticed. Therefore, a single-spore isolation was made from each culture in the following manner:

A sterile platinum needle was used to transfer spores from the stock culture to tube 1, which contained 5 cc. of distilled sterile water. From this tube a series of five dilutions was made into tubes, each containing 5 cc. of sterile distilled water. Three 3-mm. loops of material were taken from tube 1 and put into tube 2; three 3-mm. loops of material were taken from tube 2 and put into tube 3; and three 3-mm. loops of material were taken from tube 3 and put into tube 4. Tubes of standard beef agar were melted and poured into Petri dishes and allowed to cool. From each dilution tube  $\frac{1}{2}$  cc. of material was made to flow over the surface of the plated agar. Any excess material was drained off. The plates were allowed to incubate at room temperature for about 16 hours, when they were searched with a microscope for germinating spores. All microscopic examination was made through the bottom of the inverted Petri dish.

*Fusarium* spores are for the most part hyaline and to locate them on a plate of clear agar is very difficult. The scheme was devised of sprinkling a few sterile spores of *Tilletia foetans* of wheat over the surface of the agar with a tiny sterile spatula. These spores were easy to locate and gave the plane of focus in which the *Fusarium* spores could be found. The use of the sterile smut spores proves to be a great timesaver.

Usually the search for spores began with the more heavily sown plates, down through the more dilute ones until a spore was found which was sufficiently isolated so that it alone could be removed. The position of this spore was marked by a ring of India ink on the glass and it was then cut out by means of a stiff platinum cylinder illustrated in *Phytopathology* (10) and placed on the upper end of an agar slant. It was carefully watched by means of the microscope to be sure that all growth came from the spore and not from a piece of mycelium from the edge of the piece of agar, as often happened when the germinating spores were not sufficiently isolated. When it was assured that all growth came

from the spore it was considered pure and the culture was kept as a stock. If there was any question as to the source of the growth the culture was discarded. Sometimes a number of attempts had to be made before the stock culture was obtained.

#### COMPARATIVE CULTURAL STUDIES

Since the purpose of our work was the identification of the species of *Fusarium* that we had found occurring on potatoes in Montana, we began by comparing the cultural characteristics of each isolation product, hoping to be able to group together those which belonged to one species so that we might eliminate unnecessary duplication with cultures of the same species. This hope was realized only in one group. After a very few trials with different media and at different ages, one group, containing about 20 per cent of all the cultures, separated itself out very constantly. Its growth was so characteristic that we then believed and have since proved that it was *Fusarium trichothecioides* Wr. There were other groups, some seven of them, each containing from 2 to 10 cultures; but the identity of the members of the groups was not sufficiently striking to warrant leaving any of them out of further studies. However, these groupings aided materially when actual identification work began. Seventeen series of cultures have been studied. A series consisted of all the transfers made at the same time and incubated under similar conditions for the purpose of comparative studies. At least four sets of individual notes were taken on each series, so as to bring in the influence of age on the characters.

The characters emphasized in these notes were color, amount, and nature of the growth of mycelium, absence or presence and color of pseudopionnotes or sporodochia. All color determinations were based on Ridgway's "Color Standards."<sup>3</sup>

In practically all the series the cultures were inoculated in triplicate, so that when variations arose between cultures inoculated from the same stock culture a decision as to which was the more normal could be made.

As a preliminary to note taking, those cultures which had sufficient similar cultural characters to suggest identity were put into groups. Each group was designated by a letter, and these were placed in a table in parallel vertical lines, a column for each note taking, so that gross group comparisons could easily be made. Notes on each group were made in detail at the end of the table. If a culture showed only a slight variance from a group it was put into the group, but with special additional notes, and was designated in the table by the group letter with a subnumber. In so far as possible the same letters were used throughout for the same group. After a few sets of notes were taken one or two cultures which seemed most typical of a group were chosen as type cultures, and these numbers were given the same letter each time and served as the nucleus for the group represented by that letter.

Some few cultures, the number varying with the medium on which they were grown, did not develop any distinctive microscopic characters; others developed distinctive characters on certain media; and occasionally the same culture when on one medium was placed in a certain group, while on another medium it would be placed in a different group, or, as

<sup>3</sup> RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

often happened, would seem to fit none of the groups. These refractory cultures caused considerable trouble, and some of them have not yet been identified.

#### MICROSCOPIC STUDIES

##### USE OF PHOTOMICROGRAPHS

The microscopic studies that are most extensively used in classifying *Fusaria* are the size, shape, and septation of the spores. In view of the fact that different kinds of spores are produced and that in each kind there is much individual variation, the problem becomes in some cases rather complex.

In a goodly portion of the microscopic studies photomicrographs were used. The spores were mounted in water containing a small quantity of dilute Myer's flagella stain. The cover glass was blotted with a coarse filtered paper, pressed down, and sealed with paraffin to prevent evaporation and consequent movement of the spores. The pictures were taken soon after the slides were made, for the mounts gradually dried out. If they were put into a moist chamber they could sometimes be kept intact for 24 hours or more.

The Leitz horizontal photomicrographic apparatus 1A No. 398, with Leitz microscope stand, having an apochromatic condenser, was used for this work. All pictures were taken magnified 500 diameters.

The photographic method proved very convenient, for by means of it actual reproductions of spore material were made when the spores were at their best and the data were studied when convenient. This permitted a massing of data by means of which close comparative studies of microscopic characters could be made, which without photographs could not have been done.

Drawings, of course, might have been used instead, but there is no doubt that the photographic method is far superior to that of drawing. In the first place, actual reproductions of selected fields of spores are made instead of a few spores selected in accord with the personal preference of the worker and idealized and perfected in the process of drawing. Drawings are often misleading in their fine definitions and detail. Secondly, if the worker is careful to photograph an average field, which in most cases is easy to do, a great deal of additional data are recorded on the picture, which the one who was drawing would have to add in notes that take too much time when the critical stage of a large group of cultures is demanding his attention. Such data, for instance, are the percentage of spores with a certain number of septa, the percentage of macroconidia and microconidia, limits of size of different types of spores, etc.

Two complete sets of pictures were taken, except for cultures that would not fruit. One was of the fungi from cultures about 47 days old, grown on oat agar, and the other of fungi grown on lima bean agar when the cultures were about 10 days old.

The spores of some cultures would not take the stain; others, particularly those having mostly microconidia, even though perfectly sealed exhibited Brownian movement, and still other cultures produced so few spores that a field suitable to photograph was impossible to find. Many attempts, some of which were successful, were made to grow these refractory cultures on a medium that would produce a greater abundance of spores, and whenever successful pictures were taken. Notable

advance was made with some of them when grown on sterilized tomato stems and leaves.

In order to see what effect age and medium had on spore characters, three species, each typical of a group, were chosen and about 10 photographs were taken of them, grown on different media and at different ages. Our conclusions are indicated on page 354.

#### MICROSCOPIC NOTES

The only key published which includes the greater number of American species of *Fusarium* is Sherbakoff's (18), and it was therefore used as a basis for this work. In determining the various sections this key was very useful, but owing to the fine distinctions made between species and especially between varieties much difficulty was experienced in identifying an unknown culture.

In order to secure the necessary data for the identification work microscopical study was made of all cultures grown on hard oak agar and on lima bean agar, in addition to the data secured from our microphotographs of the cultures grown upon the same media, but under somewhat different conditions. The cultures grown on hard oat agar were about 15 weeks old and had been kept in the refrigerator at 10° C. The cultures grown on lima bean agar were about 4 weeks old and had been kept in a dark incubator at about 21° C.

As with the series of cultures used for the microphotographs, we had difficulty here also with certain ones not fruiting; and additional notes were made for certain cultures at about 4 weeks of age when grown on potato glucose agar and at various ages, depending upon the organism, when grown on tomato stems, potato plug, and lima bean agar.

In these examinations particular care was taken to include some mycelium in order to determine whether or not chlamydospores were present, for in the photographic work it was natural to select a field filled with spores rather than one filled with mycelium, and the presence or absence of chlamydospores, which character Sherbakoff used considerably, was often overlooked in the earlier studies.

#### MEDIA USED

A limited number of media was used in our work, for the concensus of opinion of those investigators who have used a large variety, notably Smith and Swingle (19), Apple and Wollenweber (2), and Sherbakoff (18), seems to be that little is gained from so doing. Sherbakoff believed that all important characters were brought out on hard oat agar, certain vegetable stems, tuber plugs, and potato agar containing about 5 per cent glucose. We followed the suggestion of Sherbakoff but used a few additional media. The formulas of the media follow:

**OAT AGAR.**—One hundred gm. rolled oats were put in 1,000 cc. of water and cooked for an hour in an Arnold steamer which varied in temperature from 50° to 75° C.

The product was strained through cheesecloth and the volume of liquid was brought up to 1,000 cc. Thirty gm. of agar were added, and the mixture was put in the autoclave and the pressure allowed to rise gradually to 15 pounds, where it remained for 15 minutes. The material was then tubed, and the tubes were plugged and autoclaved.

**LIMA BEAN AGAR.**—This was made in the same way as the oat agar except that the decoction was made by heating 100 gm. of broken pieces of lima beans in 1,000 cc. of water.

**POTATO GLUCOSE AGAR.**—This medium was made in the same way as the oat agar except that the decoction was made from 100 gm. of sliced potato tubers, and just before the mixture was tubed 50 gm. of glucose (T. J. Baker's c. p.) was added.

**RICE.**—About 3 gm. of rice were put into each tube, 10 cc. of tap water were added, and the tubes were plugged and autoclaved.

**POTATO PLUG.**—Cylinders cut from potato tubers were slanted and placed in tubes with enough water added to about cover the cylinder. The tubes were plugged and autoclaved.

**SWEET CLOVER STEMS.**—Stems of sweet clover (*Melilotus alba*) were cut into convenient pieces. If the stems were large enough only one was placed in a tube. Most of them, however, were small and two to four pieces were used. The stems were dry, so water enough to cover them was added. The tubes were plugged and autoclaved.

**TOMATO STEMS.**—Stems and leaves of young tomato plants (*Lycopersicon esculentum*) were cut into convenient pieces, put into tubes with distilled water added to within about  $\frac{1}{2}$  inch of the top of the stems. The tubes were plugged and autoclaved.

Five series of cultures were grown on oat agar, five on potato glucose agar, three on lima bean agar, two on potato tuber plug and one each on the other media mentioned above.

#### TEMPERATURE AND LIGHT CONDITIONS USED

By far the greater number of the series of cultures were grown in the dark in an incubator regulated between 20° and 22° C. This temperature, according to data of various investigations, seemed to be nearest the optimum for the greater number of species.

In all cases where the "dark" incubator is mentioned it refers to one the temperature of which was regulated by burning a 20-watt carbon light automatically controlled by a thermostat in the lower portion of the incubator. The cultures were kept in cans on wire screen shelves, and the light given off from this bulb should possibly be considered as influencing the results, though it does not seem probable that it did as the temperature of the room was such that the bulb was lighted but a small portion of the time.

One series of cultures grown on potato glucose agar was kept in diffused light and incubated at room temperature which varies from about 18° to 25° C.

Another series grown on potato glucose agar was inoculated in quadruplicate, and two tubes of each culture were grown in the "dark" incubator at 20° C. and two similar tubes grown in an incubator with glass doors, designated as the "light" incubator, which was in the greenhouse so placed that the cultures were in strong diffused light during the day. The tubes were kept in glass beakers and only a few in each beaker. The temperature of the "light" incubator was kept at 21.5° C.

Still another comparison between cultures grown in light and darkness was made with lima bean agar. In this case two tubes of each culture were kept in the dark incubator at 20° C. and two each were kept in diffused light in a room at a temperature which varied between 18° and 25°.

## GENERAL DISCUSSION OF METHODS

### EFFECT OF MEDIA

No distinct effort was made to determine the comparative values of different media, for in order to determine such values a large number of trials should be made on each medium under observation. However, the results which we gained from a few trials on a few media may be of some value.

**EFFECT ON CULTURAL CHARACTERS.**—The growth made on sweet clover stems lacked color. In fact certain cultures (those ordinarily grouped under K, a Discolor group, and under D, an Elegans group) which constantly produced color on all other media did not exhibit any on sweet clover. The growth was scanty in proportion to that on other media. This may have been due to the fact that the stems used were old ones which had been very dry. However, Sherbakoff (18) notes that the presence of the epidermis on stems seems to lessen the development of aerial mycelium and to favor production of fewer but better developed sporodochia. We also found that sweet clover stems seemed to favor the production of sporodochia and pseudopionnotes. A few cultures which had not at any time formed sporodochia did so on this medium. The sweet clover stems were sometimes covered with a thick, tough layer of plectenchymatic-like tissue which seemed never to bear spores.

The little experience we had with tomato stems suggested that they, too, favored the production of spores. However, our work with this medium was very limited, as we used it only on refractory cultures.

Lima bean agar proved to be another medium that did not stimulate color production. For instance on Group F (a Martiella group) notes taken 12 days after inoculation read, "a slight appearance of greenish blue growth," while cultures of the same group grown on oat at 11 days showed "various combinations of blues, greens, and purples." The D group (an Elegans group) which was mulberry purple on oat showed white or gray on lima bean.

That series of cultures (p. 350) growing on lima bean which was divided and part grown in the diffused light and part in the dark incubator showed practically no difference in color production. Only two cultures produced color in the dark that did not produce any in the light. The amount of mycelium on the lima bean cultures varied somewhat with the groups.

The mycelial growth on potato plug was abundant, and color appeared in varying degrees. That is, more color was produced on potato plugs than on lima bean or on the stems of sweet clover, but less than on the oat or potato glucose agars. Thick, tough layers of plectenchymatic tissue formed over the tuber plugs, as it did over the stems of sweet clover, but sporodochial growth was rare.

The greatest variety of colors was produced on rice, and finer group distinctions were brought out on this medium than on any other used in the cultural studies, but the colors were very mixed and they seemed not to stay true to group. Very little reliance was put on these results at the time the notes were taken. It is interesting to look back and see how nearly true to group the color determinations were. However, the mixture of colors produced was so difficult to describe that except for grouping purposes it is doubtful if rice as a cultural medium would have any specific value.



Again and again in the literature is noted the fact that for color production agar with glucose is the medium to use. Our results showed that the cultures grown on potato glucose agar did develop color, but not much more so than did those on oat agar. The color on the glucose agar in some cases was deeper than on the oat. For instance, notes taken at practically the same age on both media give for the color of group A (a Discolor group) on potato glucose "vinaceous cinnamon to orange cinnamon" and on oat it is "salmon buff to salmon color." For group C (a Discolor group) it reads "Bordeaux" on the glucose and "spinel pink" on the oat. However, the deepness of color is not constant throughout for special notes on culture No. 16 give "seashell pink" as the color on glucose and "cinnamon" as the color on oat. Our results would indicate that the two media are about equal in color production value and the amount of growth is practically the same, being abundant on both.

**EFFECT ON MICROSCOPIC CHARACTERS.**—No striking difference microscopically was noted between spores grown on lima bean, potato glucose, or oat agars (Pl. 1, A and B; 2, C). We found that the series of oat cultures kept in the refrigerator was in the best condition for spore study of all with which we worked, but it seems probable that this was due to the temperature rather than the culture medium, as the other oat series was about equal to the lima bean.

The few refractory cultures that were grown on tomato stems led us to believe that that medium might prove to be very good. At least it would be worth while to try it out further.

Appel and Wollenweber (2) concluded from their work that agar media were by no means so sure of producing normal conidia as the tubers, and the stems were found to be the most satisfactory of all. Wilcox, Link, and Pool (20) state that cultures grown on gelatin and agar media are not normal and can not be used in the determination of characters. Our results do not support these conclusions, but they confirm a statement made by Sherbakoff (18):

An agar, especially such a one as oat hard agar, often gives all the forms of fructification for these fungi, with "normal" spores and more or less typical and brilliant color production.

It might be well to add here a footnote given by Sherbakoff (18) in explanation of the variance between his results and those of Appel and Wollenweber (2):

This observation is apparently in some contradiction to the observations of Appel and Wollenweber (1910: 12-13), but indeed it is not so; because, judging by the "artificial" media actually used by them, their observations of unfitness of such media for study of "normal" growth of the *Fusaria* was based on "soft" agars too rich in sugar. The writer also found that such agars produced abnormal growth.

We found that oat agar more than any other medium used combined the qualities necessary to produce good cultural characters such as growth and color and normal spores.

#### EFFECT OF LIGHT

So little comparative work was done upon the effect of light on growth and color that our results are of rather limited value. The effect of light on *Fusarium* has never been thoroughly studied. Our conclusions and those of a few other investigators are included below.

Using lima bean agar for a medium, we found practically no difference in the amount of growth or the amount of color produced in cultures

grown in the dark incubator and those grown in diffused light, but the lima bean is not a good color producer under any condition.

With potato glucose agar there was some difference, though it was not striking. These comparisons were drawn, however, from cultures grown in the light incubator and others grown in the dark incubator (p. 350). Our notes taken when the cultures were a week old show that in the dark group A produced rufous pseudopionnotes; in the light they were ferruginous. A more intense purple color showed in group D when grown in the light than when grown in the dark. The most striking comparison, in fact practically the only striking one, was in the case of culture No. 69 which produced carmine mycelium in the light and white mycelium when grown in the dark.

Notes taken on a complete series of week-old cultures grown on potato glucose in diffused light showed practically the same results as those taken on the cultures grown in the dark incubator.

Smith and Swingle (19) found that often cultures which produced—a beautiful, rich salmon colored mycelium when grown in sunlight produced white mycelium when grown in a dark closet.

A difference in color was not noted on all media tried. Except for a difference in color these men concluded that light had no material effect on the growth.

Appel and Wollenweber (2) noted that conidia masses were much richer in color when grown in the light than in the dark. They also noted that when cultures were grown in the dark, poorly developed conidia with uneven septations and form appeared. Although direct sunlight was not exactly injurious the diffused daylight was most favorable in every way for the product of morphological characters.

#### EFFECT OF TEMPERATURE

We did no work with the effect of temperature on the fungi, but a summary of the conclusions of other workers may be of value.

In working with *Fusarium oxysporum*, Smith and Swingle (19) found that the fungus grows well on boiled potatoes at a temperature of from 15° to a little above 30° C. Below 15° the growth became slower and slower until 5° was reached, when practically no growth took place. Above 37½° no growth took place.

Link (13) did some detailed work on temperature relations of *Fusarium oxysporum* and *F. trichothecioides*. The optimum temperatures for the two are different. However, at temperatures between 15° and 20° C. a good growth was made by both.

Lewis (11) in his work with 24 cultures of *Fusaria* found that 20° to 25° C. seemed to be the best range of temperature for most of the cultures.

Appel and Wollenweber (2) in their summary of conditions which will guarantee a "normal growth" say that room temperature should be used, that is, "between 12° and 25° C. neither higher nor lower."

Edson and Shapovalov (5), working on temperature relations of six of the more common species of *Fusarium*, found that growth took place in varying amounts between 2° and 38° C. The minimum for growth was shown by *F. discolor* var. *sulphureum*, the maximum by *F. radicola*. The maximum growth for all cultures took place between 25° and 30°, though growth was abundant between 15° and 30°.

A temperature of between 19° and 22° C. was used for practically all the work reported in this paper. Incidentally it was found that cultures kept in the refrigerator at 10° showed an unusually good spore condition. It was very likely due to the fact that so low a temperature inhibited the growth and was about the optimum temperature for preservation of spores after their formation. Cultures kept in the refrigerator were always kept at room temperature for about a week after they were inoculated.

During the later spring months the light incubator in the greenhouse warmed up in the middle of the day to 30° or 35° C. We found that cultures kept in this incubator during that time deteriorated quickly.

The conclusion drawn from our own experience and that of others was that cultures can be grown as well at room temperature as at a fixed temperature, provided the temperature does not go lower than 12° or higher than 25° C. In case it is desirable to keep spores in a normal condition for a longer period of time than is possible at room temperature this can be done by keeping them in a refrigerator at 10° or less.

#### EFFECT OF AGE

Careful study of the series of cultures grown for the purpose of noting the effect of age on spore formation was in some measure disappointing. Isolated examples could be found that would illustrate practically any theory one might wish to propound. Too many factors enter in, such as moisture, nutrients, temperature, etc., for one to be able to make definite conclusions as to the effect of age. It seems that if all conditions are right to produce a "hoch"<sup>4</sup> stage of a normal culture, age does not enter in more than that a very young culture or a very old one can not produce a "Hochkultur." Only relative age then, would seem to be of importance.

#### CONCLUSIONS AND SUGGESTIONS ON METHODS

The effects of media, temperature, light, and age, though not always very great on cultural and spore characters, are sufficient to make it advisable in describing characters to describe the culture media and conditions so freely as to make the repetition of the culture upon the same medium and under approximately the same conditions easily possible, to note under what conditions these results were observed, and to keep them within certain limits.

Our experience would suggest that cultures grown on a hard oat agar, in diffuse light, and at room temperature will give the best satisfaction.

<sup>4</sup> Appel and Wollenweber (2) in their attempt to find distinctive terms by which to designate the degree of development and the age of the cultures created six terms which are briefly defined as follows:

**ANKULTUR:** A little mycelium from the original substance of some *Fusaria* is inoculated on tubers or stems. A pure, rich mycelial culture results in which there are either no conidia or only a very few, and these are apt to be irregular in shape and septation and are not suitable for morphological research.

**NORMKULTUR:** A culture which produces conidia readily and in which the spores are regular in form and septation.

**ABKULTUR:** A culture in which deterioration has set in, and the spores which have not disintegrated are small and usually have fewer septations than do those in the Normkultur. The Normkultur is subdivided into three stages:

The **JUNOKULTUR**, usually less than 8 days old, is one in which the spores have not reached a constant form of development, and spores of varying sizes and septations are found.

In the **HOCHKULTUR** the spores are truly normal, that is, comparatively even in size, shape, and septations.

In the **ALTKULTUR** the spores, due to lack of moisture or food, shrink a little in size; or if new spores are formed they are undersized, yet not deteriorated enough in form to belong to the Abkultur.

Plate 1, C and D, illustrate the "alt" and "hoch" stage of the Normkultur as seen in our work.

The cultures may be kept in a normal condition for 12 weeks or more by keeping them at a temperature of 10° C. or less.

Were the standardization work suggested on page 356 to be attempted, we would suggest as the media to be tested out a hard oat agar, potato stems, potato plugs, and possibly potato glucose agar. It would be advisable to try several regulated temperatures, together with a room temperature, the limits of which should be given, probably 12° to 25° C. Tests should be made to determine that time nearest which all the species reach the normal stage of their growth, and if possible some method should be devised to standardize moisture and humidity conditions.

#### PITFALLS IN IDENTIFICATION WORK WITH SPECIES OF FUSARIUM

The greatest obstacle in the way of accurate determination of species of *Fusarium* is the lack of a good monograph of the genus, and this lack is due in part to nonstandardization of the methods used in identification work, especially as regards kinds of media, environmental conditions, and the relative value ascribed to various characteristics of the fungus when grown in pure culture under laboratory conditions. The species and varieties intergrade and the differential characters used in the keys are not sufficiently distinct to permit any but an experienced investigator to use the key. To become an authority one must work long enough and with large enough numbers of species so that he can create within himself a conception of the species. In other words, he judges to what species the fungus in question belongs rather than actually identifying it.

Pathologists in various parts of the world often in connection with some pathological studies isolate a species of *Fusarium*. In their eagerness to name the organism which is causing economic loss they describe it so incomprehensively that future workers are not able to identify their cultures with it and therefore more new names appear.

Appel and Wollenweber (2) made a good beginning toward a monograph, but it was merely a beginning. Sherbakoff (18) has helped the situation somewhat with his "*Fusaria* of Potatoes," but his key is deserving of the criticism given above. He has split species up into so many varieties that to identify a specimen beyond its section becomes a tedious task of scientific guessing.

In the near future the botanical world, especially the mycological world, must determine and actively promote some policy with regard to trinomial nomenclature. If all the flowering plants were split into varieties on as many minor characters as are the fungi, binomial nomenclature would before long be a thing of the past. The American Code of Botanical Nomenclature (1) states in regard to categories of classification that the terms "subspecies" and "subgenus," etc., may be used when additional categories are necessary for the convenience in presentation of relationships, but "the term variety is relegated to horticultural usage."

The mycologist's difficulty arises largely from lack of perspective. The person who has worked on a single group for some time sees very real, fine distinctions which would not be at all significant to other mycologists. These distinctions may not be of enough importance to justify his making a new species, but they are too real to him to be overlooked, and he therefore originates a variety. Would it not be better to keep in mind that a species must have more or less flexible boundaries due to evolution which has taken and is now taking place,

and in describing new species or in identifying new cultures allow for a certain amount of variance? The code quoted just above defines species as "connected or coherent groups of individuals."

There is little doubt that in some cases physiological strains of species must be recognized by some system to be agreed upon, possibly by making new species, but until formally adopted by a representative body varietal names, especially those based on morphological characters, should be avoided.

#### SUGGESTION FOR A STANDARD METHOD FOR FUSARIUM STUDY

An important step in taxonomic work on *Fusarium* would be to standardize the methods for growing *Fusarium* species in somewhat the same way that certain bacteriological methods are standardized.

In order to do this it would first be necessary to carry on a comprehensive preliminary study. Interested workers in different localities would grow a large number of species of *Fusarium*, preferably subcultures from common stocks. The conditions of media composition, light, temperature, and humidity should be as nearly uniform as possible, selecting those suggested by the results of previous workers.

The method of note taking in the work should be sufficiently uniform to facilitate comparative studies of the results. From these comparative studies it could be concluded what conditions proved most satisfactory in growing species of *Fusarium*.

Selecting the most promising method thus obtained as a provisional standard, cultural work should again be carried on by a very large number of workers and with a very large number of species, and notes taken in a uniform manner. A comparison of the various notes on single species would determine whether or not the method used could be adopted as "a permanent standard."

By careful study of the various notes taken on all species of *Fusarium* used, the most stable characters could be determined and a really workable key made.

Such a procedure would involve a large expenditure of time and money and much care on the part of the workers. To find enough interested workers with the time to devote to such a study might in itself be a difficult task. However, until a key based on comprehensive data of this kind is made we see little hope for accuracy in identification. (See also page 354.)

#### RESULTS OF IDENTIFICATION WORK

The cultural and microscopic data, acquired as described above, were carefully studied and with the aid of Sherbakoff's (18) key many of the cultures under investigation were identified. Their descriptions and identifications follow according to groups. Practically all the cultures were found to be included under Wollenweber's three sections: *Elegans*, *Discolor*, and *Martiella*. Notable exceptions to this are No. 20, 69, and 75. For various reasons some few of the cultures are only provisionally identified, and two are not identified at all. The summary given in Table I will show which these are and give the reason for indecision. (See page 362.)

Since Sherbakoff's key was used, the descriptions of species given by him were taken as the standard in most cases. If questions arose about

individual characters, comparisons were made with descriptions by other investigators whenever such descriptions were available.

#### SECTION ELEGANS

One group (Group D) fell within this section. It included No. 21, 24, 25, 27, 28, 29, 31, 45, 46, 58, and 59. This was a difficult group to identify because of the scarcity of macrospores and the variations in color. Microscopically the group (with the exception of No. 59) falls into the species *Fusarium oxysporum*, but no tube culture produced sclerotia, which according to Smith and Swingle (19) were green in color and always found in cultures grown on potato plug, and according to Sherbakoff (18) were:

Bluish black in color, constantly present on potato tuber plug and sometimes on different agars.

In plate cultures grown on potato agar with 5 per cent of glucose, a few of the numbers (21, 28, and 46) produced small, dark purple spots, which on examination proved to be masses of nonsporing mycelium; but after four weeks of growth these small masses of mycelium seemed to be too loose to be called sclerotia. The fact that they did not form consistently throughout the group also suggests that they are not the sclerotia noted by the authors mentioned above.

The color of our cultures, also, does not quite agree with former descriptions of the species. Sherbakoff gives "macroconidia in mass usually of pinkish buff color" but neglects to state on what medium this is true.

On potato-glucose agar plates kept in the light most of our cultures (No. 24, 25, 27, 29, 45, 58, and 59) showed salmon coloring varying from light buff to ochraceous salmon, but shades of purple are typically found. Combinations with pinks and sometimes with greens occur but a greater or less amount of purple was characteristic of the group under all conditions. The only media used in common with Smith and Swingle were boiled rice and potato tuber plugs. Smith and Swingle found the color on the former when grown in the dark "mixed pink and lilac shading into white." Our notes show a production of purple (true) to resolane purple. On potato tuber plug these authors noted the growth when made in the darkness was "pure white changing to creamy white." We noted a slight development of a pinkish and purple pigment when grown in the dark.

However, these discrepancies in color do not seem sufficient to throw these cultures out of *Fusarium oxysporum*, but the lack of sclerotia seems important. We would, therefore, identify these cultures as *F. oxysporum* var. *asclerotium*,<sup>5</sup> a variety described by Sherbakoff which differs from *F. oxysporum*—

by the absence of sclerotia, and definite plectenchymic sporodochia, in color of the mycelium and somewhat longer and narrower macroconidia.

Sherbakoff neglects to state in what way the color differs.

Macrospores were very scarce in all the cultures of this group, and in No. 24 none at all were found. In the other numbers they varied

<sup>5</sup> It might seem inconsistent after the discussion on binomial nomenclature on page 355 to make use of varietal names in our classification. We recognize the disadvantages of trinomial nomenclature but feel justified in following it to avoid still further confusion of the names used in this genus. We feel, however, that some official recommendations and action should be taken upon the important question by societies qualified to represent mycology.

considerably in size. This variation in size seemed to bear no consistent relationship to numbers, age, media, or temperature. We are therefore including in the limits given the various sizes of the spores measured on the different cultures.

*Fusarium oxysporum* Schlecht. var. *asclerotium* Sherb. (Description taken from No. 21, 24, 25, 27, 28, 29, 31, 45, 46, and 58.)

Macroconidia typically dorsiventral, doesiventrality sometimes slight, usually distinct, and if so, ventrally curved. More or less uniform in diameter, with more or less abruptly attenuated apex; base pedicellate.

Microconidia very abundant. Oftentimes no macroconidia present, especially in cultures that have been kept for some time in stock. Chlamydospores common, mycelial intercalary and terminal, conidial intercalary.

Mycelium abundant, fine and long, from white to slight purple tint to haematoxylin violet to mulberry purple on hard oat agar grown in the dark; white to slight development of pinkish pigment and sometimes purple color on potato plug grown in dark; white to slight purple tint on potato-glucose agar grown in darkness and white to cameo pink to petunia violet when grown in the light.

Substratum colorless to purple to dull purplish black on potato-glucose agar.

The conidial measurements are as follows:

1-septate, few, 22.5 by 4.5 microns.

2-septate, rare, none measured.

3-septate, 50 to 100 per cent, 34 by 4 (22 to 52 by 3 to 5 microns).

4-septate, 0 to 40 per cent, 48 by 4.5 (35 to 60 by 4 to 5 microns).

5-septate, 0 to 20 per cent, 48 by 4.5 (35 to 60 by 4 to 5 microns).

*Fusarium sclerotioides* Sherb. var. *brevius* Sherb. (Description from No. 59.) See Plate 2, A.

Macroconidia typically dorsiventral; dorsiventrality distinct, ventrally curved, dorsally elliptic, typically broader toward apex, indistinctly pedicellate; gradually attenuated, pointed apex.

No sclerotia, no plectenchymic sporodochia.

Microconidia abundant, oval, 0- and 1-septate.

Sometimes far in excess of macroconidia.

Intercalary mycelial chlamydospores common. Mycelium well developed, white to mulberry purple on hard oat agar; white to cameo pink and petunia violet when grown in the light on potato glucose agar; slight development of pinkish pigment on potato plug. Substratum on potato glucose agar livid pink to dark maroon purple.

The conidia measurements are as follows:

1-septate rare, 22 by 4 microns.

2-septate rare, no measurements made.

3-septate 50 to 80 per cent, 36 by 5 (25 to 40 by 4.5 to 5.5) microns.

#### SECTION DISCOLOR

Three distinct groups, namely A, B, K, and one group C, which varied considerably within itself, contained species included in Wollenweber's section *Discolor*. The description of these cultures with their identification follows.

*Fusarium trichothecioides* Wr.; (Description taken from No. 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 14, 33, 38, 61, 63, 64, 76, 79, 81, 83, 91, and 97.)

Conidia not differentiated into macroconidia and microconidia, but there are two types of spores, the comma and the discolor types. The discolor type, that is conidia shaped like those of *Fusarium discolor*, is very rare. Comma type of spore slightly dorsiventral to straight, diameter more or less uniform, apex and base not differentiated rounded. (Pl. 2, B.)

Spores in powdery masses, at first on aerial mycelium, which soon covers the surface completely.

Terminal and intercalary chlamydospores occasionally noted. Mycelium abundant at first and white, soon becoming covered with powdery spore masses, which vary from pale flesh, salmon buff, chamois to buckthorn brown on potato-glucose agar; pale flesh to pale pink to pale pinkish buff on potato plug; white to pale flesh to saffron pink to light ochre to salmon buff on oat agar.

Substratum colorless to somewhat darkened on potato-glucose agar. This species is easily identified by its very characteristic powdery growth.

The conidial measurements of the comma type of spore are as follows:

0-septate, 11 by 4 (6 to 19 by 3.5 to 4.5) microns.

1-septate, 15 by 4 (12 to 24 by 3.5 to 5) microns.

2-septate, 20 by 4.5 (18 to 26 by 4 to 5) microns.

3-septate, 26 by 5 (19 to 34 by 4.5 to 6) microns.

4-septate, 32 by 5 microns.

The percentages of 0-, 1-, 2-, and 3-septate conidia vary in different cultures. In most cases 1-septate conidia predominate.

*Fusarium subpallidum* var. *roseum* Sherb. (Description taken from No. 15, 17,<sup>6</sup> 22, and 40.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, more or less uniform diameter, apex not long, rounded; base pedicellate.

Spores in small salmon-colored sporodochia, occasionally merging into pseudopionnotes. Microconidia absent. Chlamydospores sometimes found, both conidial and mycelial intercalary. Mycelium somewhat varied in color, white above to salmon orange below with some shades of purple on potato plug; flesh pink to light coral pink and rose pink with occasional slight tint of purple or yellow on potato agar with 5 per cent glucose.

Substratum, colorless to rose pink on potato agar with glucose.

The conidial measurements are as follows:

1-septate, 0 to 20 per cent, 18 by 4 microns.

2-septate, rare (no measurements made).

3-septate, 50 to 90 per cent, 25 by 4.5 (16 to 30 by 3.5 to 6) microns.

4-septate, 0 to 15 per cent, 34 by 5 microns.

5-septate, 0 to 12 per cent, 34 by 5 microns.

It is doubtful whether there is enough difference between the species *Fusarium subpallidum*, *F. subpallidum* var. *roseum*, *F. clavatum*, and *F. discolor* to warrant more than one species.

*Fusarium clavatum* Sherb. (Description taken from No. 23, 41, 42, and 43.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, slightly broader toward the apex, apex rather abruptly attenuated, base distinctly pedicellate.

Spores in small sporodochia, later merging into pseudopionnotes from pale flesh to salmon colored on oat and lima bean; light coral red to coral red on potato agar with 5 per cent glucose.

Microconidia absent.

Intercalary conidial chlamydospores sometimes present, mycelial intercalary chlamydospores occasionally found or scattered.

These cultures showed unusually close identity culturally and microscopically throughout.

The conidial measurements are as follows:

1-septate, rare.

2-septate, 2 to 6 per cent.

3-septate, 75 to 90 per cent, 27 by 4.5 (16 to 40 by 4 to 6) microns. Average limits 20 to 30 by 4 to 5 microns.

4-septate, 15 to 30 per cent, 30 by 5 microns.

5-septate, 5 to 15 per cent, 30 by 5 microns.

Practically speaking, the spore measurements and the percentages of the variously septated spores varied no more between the different media (potato glucose, oat, and lima bean agars) than between different cultures grown on the same medium.

No. 16 seems to vary between *F. clavatum* and *F. subpallidum* var. *roseum* and *F. discolor* in color characters, but the spores agree with *F. discolor* in shape and size, and sometimes in average number septations. We, therefore, are identifying it as that species.

<sup>6</sup> Practically no normal spores were found in any of the cultures of No. 17 on any of the media used. The culture seems attenuated. However, from the few spores and from earlier cultural notes we identified it with this species.



*Fusarium discolor* Ap. and Wr. var. *sulphurum* (Schlect) Ap. and Wr. (Description taken from No. 77 and 85.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, more or less uniform in diameter. Apex not long, typically slightly broader toward the apex, more or less abruptly attenuated, base distinctly pedicellate.

Spores in pseudopionnotes, flesh ochre to salmon color on all media used.

Microconidia absent.

Conidial chlamydospores often found; mycelial chlamydospores never noted, due, perhaps, to scarcity of mycelium.

Mycelium white at first, but soon becoming entirely covered by pseudopionnotes.

Substratum colorless to slight salmon coloring.<sup>7</sup>

(For photograph of No. 77, see Pl. 1, C and D.)

The conidial measurements are as follows:

2-septate, rare.

3-septate, 15 per cent, 24 by 4 (22 to 32 by 4 to 4.5) microns.

4-septate, 15 per cent, 35 by 5 microns.

5-septate, 70 per cent, 40 by 5 (35 to 45 by 4 to 5) microns.

6-septate, rare, 42 by 6 microns.

The percentages of the different septate spores vary in different cultures. For instance, 5-septate spores were 97 per cent on 7-day culture on lima bean and only 40 per cent on oat about 15 weeks old. The size also varies. The cause of difference in size would seem to depend on temperature and moisture conditions quite as much as on the medium used.

No. 84 was much the same as No. 77 and 85 but showed the following variations in spore measurements:

1-septate, rare.

2-septate, 4 per cent.

3-septate, 40 per cent, 26 by 4.5 microns.

4-septate, 30 per cent, 32 by 4.5 microns.

5-septate, 25 per cent, 36 by 5 microns.

6-septate, rare, 38 by 5 microns.

Color of growth lighter on potato glucose, orange tinge with bacterial-like growth below.

No. 52 was much the same as No. 77 and 85 but showed the following variations in spore measurements:

3-septate, 12 per cent, 25 to 40 by 4.5 microns.

4-septate, 8 per cent, 38 by 4.6 microns.

5-septate, 80 per cent, 42 by 5 (35 to 52 by 5) microns.

6-septate, rare.

Spores seem to be slightly less curved than in No. 77, though they grade into each other.

Color of growth slightly different, apricot orange rather than flesh ocre on all media used. On lima bean mycelium was medium in growth, contrasted with its scarcity in No. 77.

The darkening of the medium mentioned in note on *Fusarium discolor* var. *sulphurum* was never noticed in cultures of this number.

*F. culmorum* (W. Smith) Sacc. (Description taken from No. 84, which was the only isolation made of this species.) See Plate 2, D.

Macroconidia dorsiventral, ventrally straight or very slightly curved, slight constriction at the apical end and the pedicellate base, quite uniform diameter throughout, typically 5-septate 36 by 6 microns. 3- and 4-septate conidia are not uncommon. Conidia have thick membranes and very pronounced septa. Orange-colored sporodochia found.

Conidial chlamydospores abundant on lima bean agar, age 175 days.

On potato glucose agar mycelium abundant, bright pink above, carmine to ox-blood red below.

Substratum ox-blood red.

#### SECTION MARTIELLA

The members of the one group (F) that fell within this section were not identical in their cultural characters but were sufficiently similar to suggest a group. Microscopically it is quite easy to recognize the group

<sup>7</sup> On potato glucose agar, both in the light and in the dark, the medium sometimes darkened to a brown black and the growth became more or less powdery, from Sanford's brown to a nigger brown in color, the pseudopionnotes disappearing. Spores mounted from such cultures appeared more or less disintegrated.

Martiella, for the blunt spores are very characteristic. The size of the spores and number of septations vary considerably, and to identify the species and varieties offers many difficulties.

Sherbakoff (18), in this group also, has made varieties that could have been avoided had he made his species a little more comprehensive, and from our experience the characters of some of his species are not sufficiently stable. Such characters are comparative width and length of spores, "somewhat narrower macroconidia," "color of conidia and substratum usually paler," "frequent occurrence of bluish plectenchyma," etc.

After considerable comparative study we have identified No. 30, 35, 36, 47, 49, 51, 53, 71, 74, 80, 89, 90, 94, and 95 as *Fusarium solani*. Slight differences occur in these cultures, which, judging from a single photograph or a single set of notes, might suggest a variety of *F. solani* or of *F. Martii* or even a new species, but study of all of the data shows that these cultures do not have sufficiently stable characters to identify them as varieties or species. The cultures vary from one another and from the descriptions of *F. solani* only in minor details. Some of these variations are shown in the two photographs of *F. solani*. (Pl. 3, A and B.)

*Fusarium solani* (Mart. p. par.) Ap. et. Wr. (Description taken from No. 95 as a type.)

Macroconidia typically somewhat broader in upper half, rounded to slightly constricted apex, slightly if at all pedicellate. Normally 3-septate 28.75 by 4.5 microns (limits 27 to 38.5 by 4 to 5 microns), sometimes 2- and 4-septate, rarely 5-septate.

Pseudopionnotes and sporodochia occur commonly on most media.

Microconidia may or may not be present. When present usually abundant, round or oval in shape. Chlamydospores in mycelium terminal and intercalary, common in old cultures.

Aerial mycelium weak to well developed, typically white, neutral gray, sometimes with a purple tint.

Substratum on potato glucose agar usually from deep purplish vinaceous to dull violet black. Color on oat agar a mixture of blue, green, and purple.

*Fusarium coeruleum* (Lib.) Sacc. (Description taken from No. 55, which was the only isolation made of this species.)

Macroconidia dorsiventral, slightly ventrally curved. Basal end distinctly pedicellate. Apex rounded, more or less abruptly attenuated. Uniform diameter throughout. Three-septate spores dominant, quite variable, 31 to 42 microns by 5 to 6 microns.

Aerial mycelium feltlike in age, appressed, white to bluish white and olive buff to dusky slate, violet on potato glucose agar.

Plectenchymatic tissue and substratum on potato glucose agar violet to indigo blue and bluish black.

Chlamydospores very abundant in old cultures, terminal and intercalary and in long chains and masses.

#### OTHER SECTIONS AND UNIDENTIFIED ISOLATIONS

The few cultures that fell outside of the three sections just discussed were identified by means of Sherbakoff's key and descriptions (18), but since we did not have known cultures for comparison, no descriptions of them are included here. The identification of each as we determined it is as follows:

Section Gibbosum: No. 20, *Fusarium gibbosum*.

Section Roseum: No. 13, *F. subulatum* var. *brevius*.

Section Arthrosporiella: No. 69, *F. arthrosporioides*; No. 72, *F. anguioides*? (Chlamydospores were sometimes found.)

Section Ferruginosum: No. 75, *F. bullatum* (may be variety *roseum*).

TABLE I.—Summary of identifications made, together with source of diseased tubers, character of disease and date of isolation

Isolation No.	Source of tubers.	Date of isolation.	Suspected disease.	Determination.
1	Roundup.....	Feb. 20, 1914	Dryrot....	<i>F. trichothecioides</i> .
3	do.....	do.....	do.....	Do.
5	do.....	do.....	do.....	Do.
6	do.....	do.....	do.....	Do.
7	Stevensville.....	do.....	do.....	Do.
8	do.....	do.....	do.....	Do.
9	do.....	do.....	do.....	Do.
10	do.....	do.....	do.....	Do.
11	Lavina.....	Mar. 15, 1914	do.....	Do.
12	do.....	do.....	do.....	Do.
13	Miles City.....	Apr. 1, 1914	do.....	<i>F. subulatum</i> var. <i>brevius</i> .
14	do.....	do.....	do.....	<i>F. trichothecioides</i> .
15	Moccasin.....	Jan. 15, 1915	do.....	<i>F. subpallidum</i> var. <i>roseum</i> .
16	do.....	do.....	do.....	<i>F. discolor</i> .
17	do.....	do.....	do.....	<i>F. subpallidum</i> var. <i>roseum</i> .
20	do.....	do.....	do.....	<i>F. gibbosum</i> .
21	do.....	do.....	do.....	<i>F. oxysporum</i> var. <i>asclerotium</i> . <sup>1</sup>
22	do.....	do.....	do.....	<i>F. subpallidum</i> var. <i>roseum</i> .
23	do.....	do.....	do.....	<i>F. clavatum</i> .
24	Rockvale.....	do.....	Wilt.....	<i>F. oxysporum</i> var. <i>asclerotium</i> . <sup>1</sup>
25	do.....	Jan. 16, 1915	do.....	Do. <sup>1</sup>
27	do.....	do.....	do.....	Do. <sup>1</sup>
28	do.....	do.....	do.....	Do. <sup>1</sup>
29	do.....	do.....	do.....	Do. <sup>1</sup>
30	do.....	do.....	do.....	<i>F. solani</i> .
31	do.....	do.....	do.....	<i>F. oxysporum</i> var. <i>asclerotium</i> . <sup>1</sup>
33	Great Falls.....	Jan. 15, 1915	Dryrot....	<i>F. trichothecioides</i> .
35	Rockvale.....	Jan. 21, 1915	Wilt.....	<i>F. solani</i> .
36	do.....	do.....	do.....	Do.
38	Fallon.....	Jan. 22, 1915	do.....	<i>F. trichothecioides</i> .
40	Moccasin.....	Jan. 26, 1915	do.....	<i>F. subpallidum</i> var. <i>roseum</i> .
41	do.....	do.....	do.....	<i>F. clavatum</i> .
42	do.....	do.....	do.....	Do.
43	do.....	do.....	do.....	Do.
45	Corvallis.....	Feb. 3, 1915	do.....	<i>F. oxysporum</i> var. <i>asclerotium</i> . <sup>1</sup>
46	do.....	do.....	do.....	Do. <sup>1</sup>
47	do.....	do.....	do.....	<i>F. cF. solani</i> .
48	do.....	do.....	do.....	<i>ulmorum</i> .
49	do.....	do.....	do.....	<i>F. solani</i> .
51	Bozeman.....	June 5, 1915	Dryrot....	Do.
52	Wibaux.....	do.....	do.....	<i>F. discolor</i> var. <i>sulphureum</i> .
53	Bozeman.....	June 7, 1915	do.....	<i>F. solani</i> .
55	do.....	June 22, 1915	do.....	<i>F. coeruleum</i> .
58	Lewistown <sup>2</sup> .....	Aug. 23, 1915	Wilt.....	<i>F. oxysporum</i> var. <i>asclerotium</i> . <sup>1</sup>
59	do.....	do.....	do.....	<i>F. sclerotoides</i> var. <i>brevius</i> . <sup>1</sup>
61	Great Falls.....	Sept. 7, 1915	do.....	<i>F. trichothecioides</i> .
63	Havre.....	Dec. 10, 1915	Dryrot....	Do.
64	Roundup.....	Mar. 21, 1916	do.....	Do.
69	Billings.....	Apr. 20, 1916	do.....	<i>F. arthrosporioides</i> .
71	Darby.....	May 4, 1916	do.....	<i>F. solani</i> .

<sup>1</sup> See notes on *Elegans* group p. 357.<sup>2</sup> There is some confusion in the record of the source of this culture. It is recorded in one place as Big Arm and in another as Lewistown. Circumstantial evidence points to the latter as correct.

TABLE I.—Summary of identifications made, together with source of diseased tubers, character of disease and date of isolation—Continued

Isolation No.	Source of tubers.	Date of isolation.	Suspected disease.	Determination.
72	Bozeman.....	May 1, 1916	Dryrot.....	<i>F. anguoides</i> .
74	Havre.....	June 27, 1916	..do.....	<i>F. solani</i> .
75	Forsyth.....	Feb. 1, 1917	..do.....	<i>F. bullatum</i> .
76	....do.....	May 22, 1917	..do.....	<i>F. trichothecioides</i> .
77	....do.....	..do.....	..do.....	<i>F. discolor</i> var. <i>sulphureum</i> .
79	Stone Shack.....	..do.....	..do.....	<i>F. trichothecioides</i> .
80	Bozeman.....	..do.....	..do.....	<i>F. solani</i> .
81	Glendive.....	..do.....	..do.....	<i>F. trichothecioides</i> .
83	Billings.....	..do.....	..do.....	Do.
84	....do.....	..do.....	..do.....	<i>F. discolor</i> var. <i>sulphureum</i> .
85	....do.....	..do.....	..do.....	Do.
89	Glasgow.....	June 12, 1917	..do.....	<i>F. solani</i> .
90	Boulder.....	..do.....	..do.....	Do.
91	Hardin.....	..do.....	..do.....	<i>F. trichothecioides</i> .
94	Joliet.....	June 14, 1917	..do.....	<i>F. solani</i> .
95	Bozeman.....	June 16, 1917	..do.....	Do.
97	Circle.....	Mar. 23, 1918	..do.....	<i>F. trichothecioides</i> .

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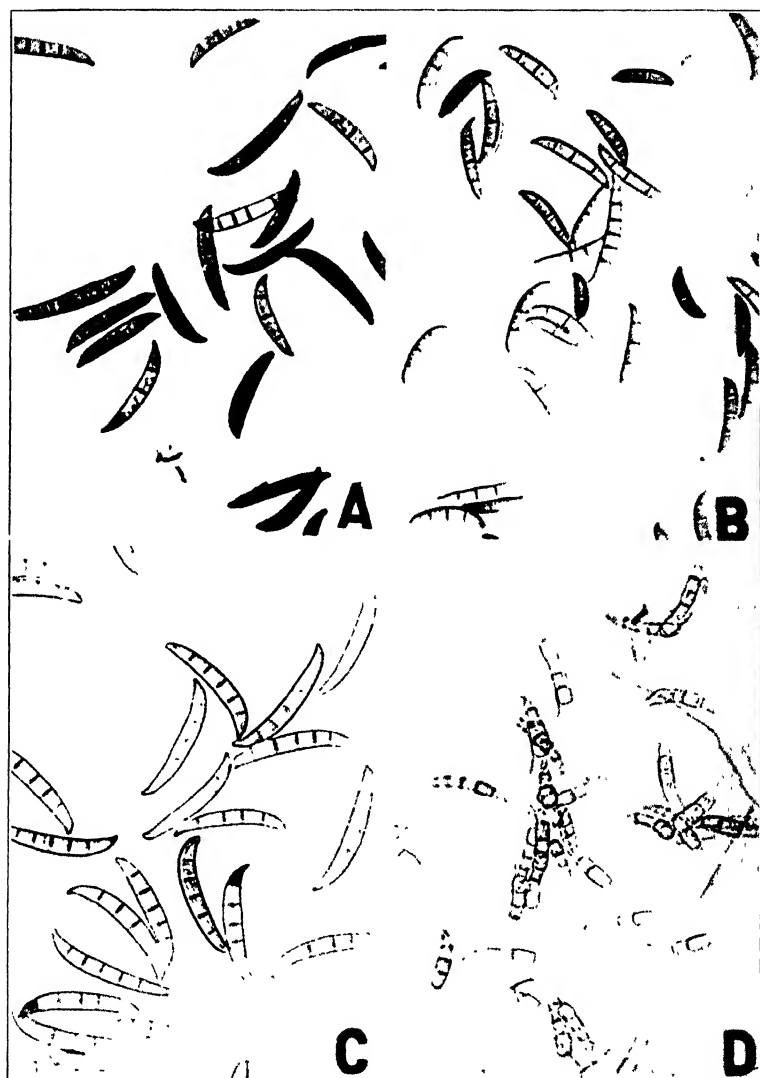
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PLATE 1

- A.—*Fusarium clavatum*, No. 41, grown on potato glucose agar. Age 43 days.  
B.—*Fusarium clavatum*, No. 41, grown on oat agar. Age 47 days.  
C.—*Fusarium discolor* var. *sulphureum*, No. 77, grown on lima bean agar. Age 7 days. (Hochkulture.)  
D.—*Fusarium discolor* var. *sulphureum*, No. 77, grown on lima bean agar. Age 91 days. (Altkulture.)





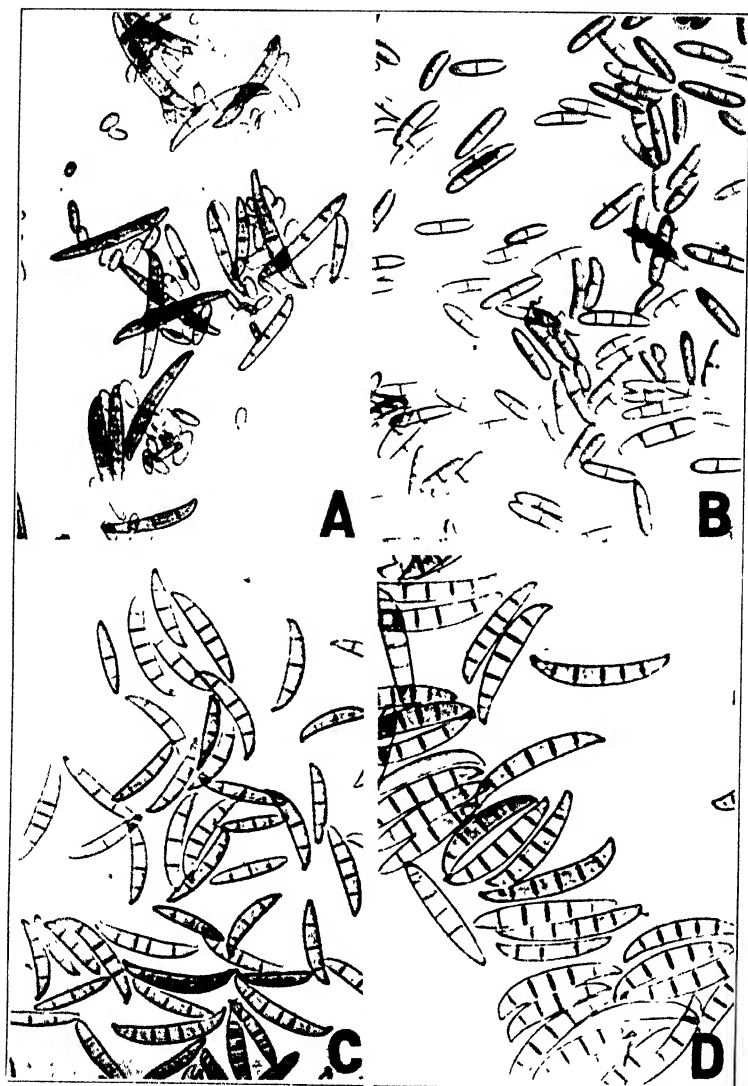


PLATE 2

- A.—*Fusarium sclerotioides* var. *brevius*, No. 59, grown on lima bean agar. Age 9 days.
- B.—*Fusarium trichothecioides*, No. 38, grown on oat agar. Age 45 days.
- C.—*Fusarium clavatum*, No. 41, grown on lima bean agar. Age 12 days.
- D.—*Fusarium culmorum*, No. 48, grown on lima bean agar. Age 10 days.

PLATE 3

- A.—*Fusarium solani*, No. 95, grown on tomato leaves and stems. Age 37 days.  
B.—*Fusarium solani*, No. 47, grown on tomato leaves and stems. Age 34 days.







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## DETERMINATION OF FATTY ACIDS IN BUTTER FAT: II <sup>1</sup>

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### INTRODUCTION

Since the publication of an earlier report (7) <sup>2</sup> on the subject, work has been continued under rather adverse conditions due to numerous changes in staff and other unavoidable interruptions. The study of technical methods for fat analysis was undertaken solely for the purpose of evolving some scheme for determining the constituents of butter fat, particularly the different fatty acids, whereby the influence of various physiological factors might be more accurately measured. Sufficient progress having been made in the methods to warrant their application, several experiments were planned with the view of obtaining information relative to the effect of breed, period of lactation, and of different oils and fats in the ration. The aim of each experiment was to determine some distinct phase of the problem, supplemental to the others, and finally to summarize all available data, as indicated by the following synopsis:

#### I. Composition of butter fat:

1. From the milk of mixed herd, grade Holsteins and grade Jerseys, fed normal rations.
2. From the milk of single animals, grade Holsteins and grade Jerseys, comparatively fresh in lactation, fed normal rations.
3. From the milk of single animals, grade Holsteins and grade Jerseys, fresh, intermediate, and late in lactation, fed normal rations.
4. From mixed milk of grade Holsteins fed a normal ration with and without the addition of various oils and fats.

II. Summary of data from Massachusetts and elsewhere, together with such general deductions as seem warranted.

It is obvious at the outset that the number of trials will be too small and too limited in scope to furnish even a tithe of the information necessary to a full understanding of the problem, but the authors hope that the investigation may at least throw some light on a difficult subject. All data, both descriptive and analytical, not deemed absolutely essential have been omitted to economize space.

### APPLICATION OF THE METHOD

The cows used in the several experiments herein reported were grade Holsteins and grade Jerseys of the experiment station herd. They were housed in comfortable, well-lighted, and well-ventilated stables

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 397-398.

and turned out into the yard for several hours every day for exercise, weather permitting. They were fed and milked twice daily, had access to water at all times, and in general received excellent care.

The cream was separated by gravity (Cooley system) and churned as sweet as possible to preserve the several milkings required. The resulting butter fat was melted, filtered, and retained for analysis in glass-stoppered bottles in a partially darkened room.

# I. COMPOSITION OF BUTTER FAT

## 1. FROM THE MILK OF MIXED HERD, GRADE HOLSTEINS AND GRADE JERSEYS, FED NORMAL RATIONS

The analysis given in Tables I and II was published in the earlier article (7), but the percentage of oleic acid has been recalculated from the iodine number of the fat instead of from that of the insoluble acids, and the results are offered as probably typical of butter fat produced by grade animals under the care and feeding practiced at the experiment station. The figures may at least serve as a tentative basis for comparison in the other experiments.

TABLE I.—Analysis of butter fat

Saponification number (s).....	(mgm.)..	231.453
Acid number (a).....	(mgm.)..	2.183
Ether number (e).....	(mgm.)..	229.270
Iodine number.....		27.999
Equivalent in oleic acid.....	(per cent) ..	31.145
Total fatty acids (T) (1.00—0.00022594 e).....	(per cent) ..	94.819
Neutralization number (n) s/T.....	(mgm.)..	244.100
Free fatty acid (A) a/n.....	(per cent) ..	.894
Soluble fatty acids (S) (T—I).....	(per cent) ..	7.319
Neutralization number.....	(mgm.)..	509.619
Insoluble fatty acids (I) by alcoholic potash.....	(per cent) ..	87.500
Neutralization number.....	(mgm.)..	221.890
Stearic acid (by crystallization).....	(per cent) ..	13.010
Glycerol (0.00054703 e).....	(per cent) ..	12.542

TABLE II.—Fatty acids in butter fat

Fatty acids.	Per cent
Soluble acids:	
Butyric acid (by difference).....	3.153
Caproic acid.....	1.360
Caprylic acid.....	.975
Capric acid.....	1.831
Total.....	7.319
Insoluble acids:	
Lauric acid.....	6.895
Myristic acid.....	22.618
Palmitic acid (by difference).....	15.458
Stearic acid.....	11.384
Oleic acid.....	31.145
Total.....	87.500
Total fatty acids.....	94.819

As decomposition vitiates certain determinations in the analysis of oils and fats, the results fail to create a perfect entity, but with experienced workers such errors may gradually be reduced to a minimum.

## 2. FROM THE MILK OF SINGLE ANIMALS, GRADE HOLSTEINS AND GRADE JERSEYS, COMPARATIVELY FRESH IN LACTATION, FED NORMAL RATIONS

The cows in this test were fair types of their respective breeds and comparable in age. Cecile II had freshened more recently than the others. The Holsteins were a high-fat strain of moderate milk yield. The milk produced was the daily average for the period in which cream was saved for churning. The milk analyzed was a five-days' composite, taken about the same time as the cream samples. The feeds, both grain and roughage, were average products of their kind of which analyses were not considered necessary. The results of this test are given in Table III.

TABLE III.—Records of cows and milk analysis

	Colantha II.	Samantha II.	Cecile II	Peggy
Breed.....	Grade Holstein....	Grade Holstein....	Grade Jersey. ....	Grade Jersey.
Date of birth.....	Oct. 14, 1914 .....	Aug. 18, 1909 .....	Dec. 18, 1912 .....	... 1910.
Last calf dropped ..	July 3, 1917 .....	Aug. 16, 1917 .....	Oct. 19, 1917 .....	Aug. 9, 1917.
Condition on calving ..	Good flesh.....	Good flesh .....	Good flesh .....	Good flesh .....
Date served .....	Oct. 22, 1917 .....	Oct. 26, 1917 .....	Nov. 24, 1917 .....	Nov. 8, 1917.
Weight of animal .....	1,000 pounds. ....	1,192 pounds .....	700 pounds (?) .....	780 pounds.
Daily ration:				
Hay.....	18 pounds .....	24 pounds. ....	7 pounds .....	17 pounds.
Alfalfa.....	.....	.....	4 5 pounds. ....	.....
Corn stover .....	.....	.....	16 .....	.....
Gluten feed .....	4 pounds. ....	4 5 pounds. ....	3 8 pounds .....	3 pounds.
Wheat bran .....	.....	.....	2 5 pounds. ....	.....
Ground oats .....	2 pounds .....	2 5 pounds .....	.....	1 pound.
Corn bran .....	4 pounds .....	4 pounds .....	.....	4 pounds.
Milk produced (daily average). <sup>1</sup>	Oct. 22 to 25, 1917, 23.7 pounds	Oct. 26 to 29, 1917, 29.7 pounds.	Nov. 5 to 8, 1917, 24 3 pounds.	Oct. 18 to 21, 1917, 17.6 pounds
Milk analysis:				
Solids (gravimetric) ..	13 38 per cent.	12.95 per cent.	14.43 per cent.	15 82 per cent.
Fat (Babcock) .....	4.40 per cent.	4 55 per cent.	5 40 per cent.	6 00 per cent.
Proteids (N X 6 25) ..	3 37 per cent.	3 22 per cent.	3 40 per cent.	3 98 per cent.
Lactose (by difference)	4 87 per cent.	4 47 per cent.	4 92 per cent.	4 45 per cent.
Ash. ....	.74 per cent.	.71 per cent.	.71 per cent.	.79 per cent.

<sup>1</sup> For the days cream was saved for churning.

## ANALYSIS OF BUTTER FAT

The methods employed for the ordinary analysis of butter fat have been described previously (8). The use of glycerol potash for the determination of insoluble acids and the preparation of stock has been superseded, however, by alcoholic potash as less drastic in its action on the unsaturated acids; but even the latter under careful manipulation tends to induce intramolecular changes resulting in a loss of iodine absorption and to some extent an increase in alkali-consuming power due to instability of the resulting molecule, particularly with linolic, linolenic, and other highly unsaturated acids, as shown by Fittig, Varrentrapp, and Schrauth, summarized in another article (10, p. 362). Recent experiments, although incomplete, indicate that under control conditions such decomposition can be largely prevented with normal butter fat. The determination of stearic acid by crystallization from alcohol is unquestionably simple in theory but rather difficult in practice except in a cold, dry atmosphere, since condensed moisture is a vitiating factor. The analysis of butter fat is given in Table IV.



TABLE IV.—Analysis of butter fat

	Colantha II.	Samantha II.	Cecile II.	Peggy.
Saponification number (s).....mgm..	229.466	230.324	230.907	230.968
Acid number (a).....mgm..	.927	2.386	1.626	3.657
Ether number (e).....mgm..	228.539	227.938	229.281	227.311
Iodin number.....	30.622	29.605	28.159	22.720
Equivalent in oleic acid.... per cent..	34.063	32.931	31.323	25.273
Total fatty acids (T) (1.00—0.00022594 e)..... per cent..	94.836	94.850	94.820	94.864
Neutralization number (n) s/T..... mgm..	241.961	242.830	243.521	243.473
Free fatty acids (A) a/n.... per cent..	.383	.983	.668	1.502
Soluble fatty acids (S) (T—I)..... per cent..	6.470	7.023	7.226	6.510
Neutralization number..... mgm..	503.184	513.356	517.409	495.637
Insoluble fatty acids (I) by alcoholic potash..... per cent..	88.366	87.827	87.594	88.354
Neutralization number.... mgm..	222.835	221.197	220.927	224.893
Stearic acid by crystallization..... per cent..	13.709	16.997	20.321	13.398
Glycerol (0.00054703 e)..... per cent..	12.502	12.469	12.542	12.435

The percentages of total fatty acids were 94.843 in the Holsteins and 94.842 in the Jerseys and their neutralization numbers 242.396 and 243.497 mgm., respectively. The percentages of free fatty acids were 0.683 and 1.085, indicating a greater tendency to hydrolyze in the Jerseys. The percentages of soluble fatty acids were 6.747 and 6.868, and their neutralization numbers 508.270 and 506.523. The percentages of insoluble fatty acids were 88.097 and 87.974, and their neutralization numbers 222.016 and 222.910. The nature of these differences is apparent from the data, but the extent can not be actually apportioned with five components involved. The two breeds showed a remarkably close agreement in the several groups of fatty acids and of glycerol, but the proportion of high molecular weight acids in the insolubles was slightly more pronounced in the Holsteins.

The composition of neither breed conformed particularly to that of the herd sample (Table I), although the difference in actual percentage of soluble and of insoluble acids was more appreciable than the difference in proportion of constituent acids in each group, as indicated by the neutralization numbers.

#### ESTERIFICATION PROCESS

The method (7) of esterification, purification, and fractionation of the ethyl esters remains substantially as published. Attention might be called, however, to some minor modifications that have since been adopted. Alcohol for esterification is prepared by distilling approximately 2 liters in a water bath over 600 to 700 gm. of granulated caustic lime (95 per cent CaO) and 30 to 40 gm. of yellow ceresin wax and redistilling over fresh lime and ceresin until free from water, as indicated by the absence of lime from solution. On the final distillation the first and last portions are rejected for additional treatment and the main portion is preserved. This process has proved the most reliable so far tested. Three cc. of concentrated sulphuric acid have been substituted as a catalyzer in place of dry hydrochloric acid at a material saving in time and convenience and considerable gain in efficiency.

A second extraction of the alcoholic residue and combined washings with ether is no longer considered necessary. A "high" side tube distillation flask is employed in both the preliminary distillation of the esters and the fractionation. The number of fractions has been increased to 7. Fraction 1, ranging to about 100° C.<sup>3</sup>, is the most volatile portion derived from the esters, largely ether but containing considerable ethyl butyrate. It is made to a volume of 500 cc. with ether and the alkali-consuming power of an aliquot is determined; from this the grams of ethyl butyrate can be calculated. Fractions 2 to 6 are now largely determined by weight, a small Troemner scale with prepared counterpoise being used, since the temperature uncorrected and influenced by the speed of distillation has not proved entirely a safe guide. The content of ethyl oleate is no longer considered a prominent factor. By using an adapter on the end of the condenser little difficulty is encountered in changing flasks. The recovery in fractions 2 to 7 has been greatly increased and now averages 185 gm. from 300 gm. of butter fat, with a maximum of 216 gm. thus far.

TABLE V.—Weight and analysis of fractions (ethyl esters)

COLANTHA II					
Fraction No	Range of fraction.	Weight of fraction	Saponification number.	Iodin number.	Ethyl oleate
	° C.	Gm	Mgm.		Per cent.
1.....	± 70 to 90	.....	<sup>a</sup> 1,453.197	.....	.....
2.....	90 to 165	5.6093	396.205	3.776	4.618
3.....	165 to 217	3.9590	337.924	7.241	8.854
4.....	217 to 257	6.4436	267.689	11.872	14.518
5.....	257 to 286	13.7516	238.602	14.216	17.384
6.....	286 to 317	57.4804	218.942	16.275	19.902
7.....	317 to 332	97.4166	200.959	22.324	27.299
Total.....	.....	184.6605	.....	.....	.....

SAMANTHA II					
1.....	± 70 to 90	.....	<sup>a</sup> 1,655.186	.....	.....
2.....	90 to 166	5.5964	385.988	5.055	6.182
3.....	166 to 218	3.9766	335.668	8.944	10.937
4.....	218 to 258	5.4594	270.757	12.834	15.604
5.....	258 to 286	14.2372	238.847	14.605	17.860
6.....	286 to 317	53.7426	217.020	16.714	20.439
7.....	317 to 327	79.3705	200.880	19.797	24.209
Total.....	.....	162.3827	.....	.....	.....

CECILE II					
1.....	± 70 to 100	.....	<sup>a</sup> 2,084.412	.....	.....
2.....	100 to 170	5.6765	405.373	2.048	2.504
3.....	170 to 220	4.0712	357.188	3.725	4.555
4.....	220 to 261	4.6266	287.805	7.613	9.309
5.....	261 to 286	8.5821	240.297	10.207	12.481
6.....	286 to 318	55.7372	218.974	14.914	18.238
7.....	318 to 326	71.7496	204.804	19.707	24.098
Total.....	.....	150.4432	.....	.....	.....

<sup>a</sup> Total alkali-consuming power of the fraction.

<sup>b</sup> With a minimum of 100° C. the complete elimination of ether and alcohol is more definitely assured; otherwise these might vitiate fraction 2.

TABLE V.—Weight and analysis of fractions—Continued

## PEGGY

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	° C.	Gm.	Mgm.		Per cent.
1. ....	± 70 to 90	.....	<sup>a</sup> 1,239.987	.....	.....
2. ....	90 to 167	4.5351	412.882	2.279	2.787
3. ....	167 to 220	4.3029	343.102	5.383	6.583
4. ....	220 to 260	5.7014	273.896	9.093	11.119
5. ....	260 to 286	14.9828	240.286	11.265	13.775
6. ....	286 to 317	54.2113	218.762	12.135	14.839
7. ....	317 to 328.5	92.9064	203.218	14.813	18.114
Total .....		176.6399	.....	.....	.....

<sup>a</sup> Total alkali-consuming power of the fraction.

TABLE VI.—Fatty acids in butter fat

Fatty acids.	Colantha II	Samantha II.	Cecile II.	Peggy.
	Per cent.	Per cent.	Per cent.	Per cent.
Soluble acids:				
Butyric acid (by difference).....	2.260	2.928	3.007	2.726
Caproic acid.....	1.588	1.688	1.794	1.290
Caprylic acid.....	.648	.744	.881	.779
Capric acid.....	1.974	1.663	1.544	1.715
Total .....	6.470	7.023	7.226	6.510
Insoluble acids:				
Lauric acid .....	7.618	6.317	5.616	6.598
Myristic acid .....	19.768	17.455	20.534	21.782
Palmitic acid (by difference).....	14.803	16.196	12.321	22.863
Stearic acid .....	12.114	14.928	17.800	11.838
Oleic acid.....	34.063	32.931	31.323	25.273
Total .....	88.366	87.827	87.594	88.354
Total fatty acids.....	94.836	94.850	94.820	94.864

The percentage of butyric acid was 2.594 in the Holsteins and 2.867 in the Jerseys; of caproic acid, 1.638 and 1.542; of caprylic acid, 0.696 and 0.830; and of capric acid, 1.819 and 1.630. The differences were compensating, the high percentages alternating, but were not sufficiently indicative to warrant deductions. The percentage of lauric acid was 6.968 in the Holsteins and 6.107 in the Jerseys; of myristic acid, 18.612 and 21.158; of palmitic acid, 15.500 and 17.592; of stearic acid, 13.521 and 14.819; and of oleic acid, 33.497 and 28.298. The outstanding feature was the low oleic acid content of Peggy's fat, which necessarily affected the results. Despite this fact, however, a lower percentage of myristic and stearic acids and a higher percentage of oleic acid appeared to be characteristic of the Holsteins as contrasted with the Jerseys.

As compared with the herd sample (Table II) neither breed showed any appreciable differences to which attention has not already been called. That the percentage of caproic acid in the herd sample was apparently abnormal, being noticeably lower than is indicated by a subsequent average, is taken into consideration.

### 3. FROM THE MILK OF SINGLE ANIMALS, GRADE HOLSTEINS AND GRADE JERSEYS, FRESH, INTERMEDIATE, AND LATE IN LACTATION, FED NORMAL RATIONS

The cows were grades, the Jerseys on the average were appreciably older, but all had freshened within a few weeks. Cecile II was the same cow used the year previous. The Holsteins were a 4 per cent strain of fair milk yield. Fancy III, although a high-grade Jersey, did not produce as rich milk as many of that breed.

In the sampling of milk and cream there was an interval of four months<sup>4</sup> between the so-called fresh and intermediate periods of lactation and an additional three months<sup>4</sup> to the late period, during which time the milk yield decreased nearly 32 per cent in the Holsteins and 28 per cent in the Jerseys, and in general the percentages of solids and fat increased but not as consistently as might have been expected.

The same grain mixture and similar hay were fed throughout the different lactation periods, but the amounts varied as stated. Analysis of the hay and various grains indicates their quality (Table X).

TABLE VII.—Records and analysis of milk from cows fresh in lactation

	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Breed	Grade Holstein	Grade Holstein	Grade Jersey	Grade Jersey
Date of birth	Nov. 16, 1913	Aug. 25, 1914	Dec. 18, 1912	Aug. 11, 1908.
Last calf dropped	Aug. 20, 1918.	Oct. 6, 1918.	Aug. 26, 1918	Oct. 12, 1918.
Condition on calving	Good flesh	Good flesh	Thin	Thin
Date served	Dec. 9, 1918	Nov. 15, 1918	Jan. 9, 1919	Mar. 26, 1919
Weight of animal	1,195 pounds	1,113 pounds	713 pounds	863 pounds.
Daily ration				
Hay	26 pounds	24 pounds	18 pounds	22 pounds.
Grain mixture	12 pounds	12 pounds.	8 pounds	9 pounds.
Cottonseed meal, 30 per cent.				
Gluten feed, 30 per cent.				
Wheat bran, 30 per cent.				
Corn feed meal, 30 per cent.				
Milk produced (daily average) <sup>1</sup>	Sept. 23 to 26, 1918, 33.5 pounds.	Oct. 31 to Nov. 3, 1918, 41.4 pounds.	Sept. 23 to 27, 1918, 23.7 pounds.	Oct. 31 to Nov. 3, 1918, 33.5 pounds.
Milk analysis:				
Solids (gravimetric)	12.47 per cent.	12.80 per cent	14.43 per cent.	12.50 per cent.
Fat (Babcock)	3.93 per cent	4.38 per cent.	5.20 per cent	4.18 per cent.
Proteids (N×6.25)	3.12 per cent	3.02 per cent	3.66 per cent	3.06 per cent.
Lactose (by difference)	4.69 per cent	4.69 per cent.	4.85 per cent	4.31 per cent.
Ash	.73 per cent	.71 per cent.	.72 per cent	.75 per cent.

<sup>1</sup> For the days cream was saved for churning.

TABLE VIII.—Records and analysis of milk from cows intermediate in lactation

	Colantha	Samantha IV.	Cecile II.	Fancy III.
Weight of animal	1,280 pounds	1,163 pounds	753 pounds.	878 pounds.
Daily ration:				
Hay	24 pounds	22 pounds	18 pounds	20 pounds
Grain mixture	13 pounds	13 pounds	8 pounds	10 pounds.
Milk produced (daily average) <sup>1</sup>	Jan. 26 to 29, 1919, 27.9 pounds.	Jan. 30 to Feb. 2, 1919, 33.6 pounds.	Jan. 26 to 29, 1919, 16.7 pounds.	Jan. 30 to Feb. 2, 1919, 29.8 pounds.
Milk analysis:				
Solids (gravimetric)	12.84 per cent.	12.66 per cent.	15.53 per cent.	13.00 per cent.
Fat (Babcock)	4.28 per cent	4.25 per cent.	6.13 per cent.	4.80 per cent.
Proteids (N×6.25)	3.32 per cent.	3.07 per cent.	4.10 per cent.	3.10 per cent.
Lactose (by difference)	4.52 per cent.	4.68 per cent.	4.58 per cent.	4.40 per cent.
Ash	.75 per cent	.66 per cent.	.71 per cent.	.70 per cent.

<sup>1</sup> For the days cream was saved for churning.

<sup>4</sup> Approximate length of period

TABLE IX.—Records and analysis of milk from cows late in lactation

	Colantha.	Samantha IV	Cecile II.	Fancy III.
Weight of animal	1,336 pounds	1,196 pounds	730 pounds	899 pounds.
Daily ration:				
Hay	22 pounds	22 pounds	16 pounds	20 pounds
Grain mixture	13 pounds	13 pounds	8 pounds	10 pounds.
Milk produced (daily average). <sup>1</sup>	Apr. 23 to 30, 1919, 23.5 pounds	Apr. 19 to 25, 1919, 27.7 pounds.	Apr. 19 to 23, 1919, 16.6 pounds.	Apr. 21 to 27, 1919, 25.1 pounds.
Milk analysis:				
Solids (gravimetric)	13.45 per cent	13.12 per cent.	15.07 per cent.	13.00 per cent.
Fat (Babcock)	4.65 per cent	4.40 per cent.	5.80 per cent	4.80 per cent.
Proteids (N×6.25)	3.52 per cent	3.18 per cent	3.93 per cent	3.27 per cent.
Lactose (by difference)	4.54 per cent	4.85 per cent.	4.64 per cent	4.19 per cent.
Ash	.74 per cent	.69 per cent	.70 per cent	.74 per cent.

<sup>1</sup> For the days cream was saved for churning.

TABLE X.—Analysis of feeds

	Hay.	Cottonseed meal.	Gluten feed	Wheat bran.	Corn feed meal.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Moisture	12.35	7.50	9.32	11.90	16.70
Dry matter:					
Ash	6.03	6.06	4.08	6.93	1.55
Protein (N×6.25)	8.09	38.51	26.77	16.44	10.31
Fiber	30.16	14.18	8.49	11.84	2.38
Extract matter	53.07	34.10	56.81	59.31	84.27
Fat	2.65	7.15	3.85	5.48	1.49

TABLE XI.—Analysis of butter fat from cows fresh in lactation

	Colantha.	Samantha IV	Cecile II.	Fancy III.
Saponification number (s) (mgm.)	232.561	233.016	232.456	235.333
Acid number (a) (mgm.)	2.850	1.663	1.997	2.803
Ether number (e) (mgm.)	229.711	231.353	230.459	232.530
Iodin number	30.135	28.285	26.900	25.477
Equivalent in oleic acid (per cent)	33.521	31.463	29.922	28.340
Total fatty acids (T) (1.00—0.00022594 e)				
Neutralization number (n) (s/T)	94.810	94.773	94.793	94.746
(mgm.)	245.292	245.867	245.225	248.383
Free fatty acids (A) a/n (per cent)	1.162	.676	.814	1.128
Soluble fatty acids (S) (T—I) (per cent)	7.668	8.008	7.382	8.601
Neutralization number (mgm.)	513.641	505.132	509.428	510.662
Insoluble fatty acids (I) by alcoholic potash (per cent)	87.142	86.765	87.411	86.145
Neutralization number (mgm.)	221.678	221.939	222.913	222.196
Stearic acid by crystallization (per cent)	17.399	18.669	23.304	20.991
Glycerol (0.00054703 e) (per cent)	12.566	12.656	12.607	12.720

The percentages of total fatty acids were 94.792 in the Holsteins and 94.770 in the Jerseys and their neutralization numbers 245.580 and 246.804 mgm., respectively, indicating a slightly smaller proportion of high molecular weight acids in the Jerseys due to Fancy III's fat. The

percentages of free fatty acids were 0.919 and 0.971. The percentages of soluble fatty acids were 7.838 and 7.992, and their neutralization numbers 509.387 and 510.045, respectively. The percentages of insoluble fatty acids were 86.954 and 86.778, and their neutralization numbers 221.809 and 222.555. The percentage was exceptionally low in Fancy III's fat. The percentages of glycerol were 12.611 and 12.664.

The two breeds contained similar quantities of the several groups of fatty acids and of glycerol, and their acid mixtures, both soluble and insoluble, were of like character, although the neutralization number of the insoluble was slightly higher in the Jerseys.

Both breeds exceeded the herd sample (Table I) materially in soluble fatty acids.

TABLE XII.—Analysis of butter fat from cows intermediate in lactation

	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Saponification number ( <i>s</i> ).....(mgm.)..	229.741	229.190	231.781	234.107
Acid number ( <i>a</i> ).....(mgm.)..	1.646	2.029	2.728	1.732
Ether number ( <i>e</i> ) .. .. .(mgm.)..	228.095	227.161	229.053	232.375
Iodin number .. .. .(mgm.)..	31.501	31.189	24.382	26.750
Equivalent in oleic acid... (per cent)...	35.040	34.693	27.122	29.756
Total fatty acids ( <i>T</i> ) (1.00—0.00022594 <i>e</i> ) ..	94.846	94.868	94.825	94.750
Neutralization number ( <i>n</i> ) .. .. .(mgm.)..	242.225	241.588	244.430	247.079
Free fatty acids ( <i>A</i> ) <i>a/n</i> (per cent) ..	.680	.840	1.116	.701
Soluble fatty acids ( <i>S</i> ) ( <i>T</i> — <i>I</i> ) (per cent)...	6.744	6.703	6.773	7.542
Neutralization number .. .. .(mgm.)..	535.083	539.370	527.122	538.345
Insoluble fatty acids ( <i>I</i> ) by alcoholic potash .. .. .(per cent)...	88.102	88.165	88.052	87.208
Neutralization number.....(mgm.)..	219.808	218.949	222.687	221.889
Stearic acid by crystallization.....(per cent)...	16.923	17.732	20.493	18.287
Glycerol (0.00054793 <i>e</i> ).....(per cent)...	12.477	12.426	12.530	12.712

The percentages of total fatty acids were 94.857 in the Holsteins and 94.788 in the Jerseys, and their neutralization numbers 241.907 and 245.755 mgm., respectively. The percentages of free fatty acids were 0.760 and 0.908. The percentages of soluble fatty acids were 6.724 and 7.158, and their neutralization numbers 537.227 and 532.734, which were considerably higher than usual. The percentages of insoluble fatty acids were 88.134 in the Holsteins and 87.630 in the Jerseys, and their neutralization numbers 219.379 and 222.288. The percentages of glycerol were 12.452 and 12.621, a noticeable difference.

The Holsteins exceeded the Jerseys in total and insoluble fatty acids and contained a considerably higher proportion of high molecular weight acids in the insolubles. The Jerseys suffered a smaller loss of soluble acids and of glycerol.

The Holsteins exceeded the herd sample (Table I) in insoluble acids with a corresponding loss of soluble acids and a material loss in neutralization number of the insolubles. The Jerseys averaged substantially the same as the herd sample.

TABLE XIII.—Analysis of butter fat from cows late in lactation

	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Saponification number (s).....(mgm.)	224.626	225.709	230.834	231.259
Acid number (a).....(mgm.)	2.440	2.701	3.161	2.432
Ether number (e).....(mgm.)	222.186	223.008	227.673	228.827
Iodin number.....	36.241	33.649	26.952	29.472
Equivalent in oleic acid.....(per cent)	40.313	37.430	29.980	32.783
Total fatty acids (T) (1.00—0.00022594 e).....(per cent)...	94.980	94.961	94.856	94.830
Neutralization number (n) (s/T).....(mgm.)	236.498	237.686	243.352	243.867
Free fatty acid (A) a/n.....(per cent)	1.032	1.136	1.299	.997
Soluble fatty acids (S) (T—A).....(per cent)	6.509	6.577	7.232	7.526
Neutralization number.....(mgm.)	519.511	519.340	516.081	527.106
Insoluble fatty acids (I) by alcoholic potash.....(per cent)	88.471	88.384	87.624	87.304
Neutralization number.....(mgm.)	215.676	216.727	220.842	219.450
Stearic acid by crystallization.....(per cent)	21.628	20.399	21.033	21.512
Glycerol (0.00054703 e).....(per cent)	12.154	12.199	12.454	12.518

The percentages of total fatty acids were 94.971 in the Holsteins and 94.843 in the Jerseys, and their neutralization numbers 237.092 and 243.610 mgm., respectively. The percentages of free fatty acids were 1.084 and 1.148. The percentages of soluble fatty acids were 6.543 and 7.379, and their neutralization numbers 519.426 and 521.594, which were appreciably high. The percentages of insoluble fatty acids were 88.428 and 87.464, and their neutralization numbers 216.202 and 220.146. The percentages of glycerol were 12.177 and 12.486, which was decidedly low in the Holsteins.

The Holsteins further exceeded the Jerseys in total and insoluble fatty acids, with a still greater difference in neutralization number of the insolubles. The Jerseys maintained a high soluble acid content and only a moderate loss of glycerol.

The Holsteins exceeded the herd sample (Table I) in total and insoluble fatty acids by a larger amount than in the previous period and with a greater loss in neutralization number of the insolubles and of glycerol. The Jerseys again averaged practically the same as the herd sample except for an appreciable loss in neutralization number of the insolubles.

TABLE XIV.—Weight and analysis of fractions of milk from cows fresh in lactation (ethyl esters)

## COLANTHA

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	° C.	Gm.	Mgm.		Per cent.
1.....	± 70 to 85	.....	a 1,472.835	.....	.....
2.....	85 to 145	5.6846	398.375	2.098	2.565
3.....	145 to 190	5.1990	361.366	4.514	5.520
4.....	190 to 230	3.8515	283.442	10.292	12.586
5.....	230 to 278	13.9373	242.332	14.268	17.448
6.....	278 to 310	57.5422	214.019	19.080	23.331
7.....	310 to 328.5	128.4525	197.332	26.687	32.634
Total.....	.....	214.6671	.....	.....	.....

a Total alkali-consuming power of the fraction

TABLE XIV.—Weight and analysis of fractions of milk from cows fresh in lactation (ethyl esters)—Continued

## SAMANTHA IV (260.1 GM. OF FAT)

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1.....	±70 to 80	.....	<sup>a</sup> 1,329.760	.....	.....
2.....	80 to 141	4.2115	391.789	3.229	3.948
3.....	141 to 186	4.9620	377.169	4.997	6.111
4.....	186 to 224	3.6875	290.948	8.754	10.705
5.....	224 to 271	10.8334	242.858	12.122	14.823
6.....	271 to 306	45.2200	216.148	15.072	18.430
7.....	306 to ?	87.4847	199.026	20.981	25.656
Total...		156.3001			

## CECILE II (261.96 GM. OF FAT)

1.....	±70 to 80	.....	<sup>a</sup> 1,085.690	.....	.....
2.....	80 to 144	4.6898	397.378	2.622	3.206
3.....	144 to 191	5.0742	345.943	4.517	5.523
4.....	191 to 220	3.7697	279.378	7.469	9.133
5.....	220 to 259	11.5881	242.983	10.301	12.596
6.....	259 to 310	47.8509	214.861	14.929	18.256
7.....	310 to 325	96.3112	197.850	21.972	26.868
Total.....		169.2839			

## FANCY III

1.....	±70 to 82	.....	<sup>a</sup> 1,822.107	.....	.....
2.....	82 to 125	5.9548	414.095	2.389	2.921
3.....	125 to 155	5.6521	376.234	3.962	4.845
4.....	155 to 192	4.1875	295.894	7.721	9.441
5.....	192 to 254	13.4642	244.204	11.283	13.797
6.....	254 to 298	55.5961	215.916	14.377	17.581
7.....	298 to 314	104.4243	199.966	19.299	23.599
Total.....		189.2790			

<sup>a</sup> Total alkali-consuming power of the fraction.

TABLE XV.—Fatty acids in butter fat from cows fresh in lactation

Fatty acids.	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Soluble acids:	Per cent.	Per cent.	Per cent.	Per cent.
Butyric acid (by difference).....	3.179	.....	2.998	4.230
Caproic acid.....	2.210	.....	1.857	2.400
Caprylic acid.....	.778	.....	1.030	.714
Capric acid.....	1.501	.....	1.497	1.257
Total.....	7.668	8.008	7.382	8.601
Insoluble acids:				
Lauric acid.....	4.949	4.831	4.533	4.804
Myristic acid.....	20.129	18.445	19.097	20.746
Palmitic acid (by difference).....	13.381	15.828	13.419	14.172
Stearic acid.....	15.162	16.198	20.370	18.083
Oleic acid.....	33.521	31.463	29.992	28.340
Total.....	87.142	86.765	87.411	86.145
Total fatty acids.....	94.810	94.773	94.793	94.746



The percentage of butyric acid was 3.179 in the Holsteins (1 sample) and 3.614 in the Jerseys; of caproic acid, 2.210 and 2.129; of caprylic acid, 0.778 and 0.872; and of capric acid, 1.501 and 1.377. The differences were not pronounced, but there was a tendency toward a higher content of butyric and caprylic acids in the Jerseys.

The percentage of lauric acid was 4.890 in the Holsteins and 4.669 in the Jerseys; of myristic acid, 19.287 and 19.922; of palmitic acid, 14.605 and 13.796; of stearic acid, 15.680 and 19.227; and of oleic acid, 32.492 and 29.166. The percentage of myristic and stearic acids was lower and that of oleic acid higher in the Holsteins than in the Jerseys.

The soluble acids of the Holsteins were of the same general character as those of the herd sample (Table II), differing somewhat in total but more particularly in the amount of each acid in the mixture, although such differences were largely compensating. This was equally true of the insolubles. In the Jerseys these differences were even more marked than in the Holsteins.

TABLE XVI.—*Weight and analysis of fractions of milk from cows intermediate in lactation (ethyl esters)*

COLANTHA

Fraction No.	Range of fraction	Weight of fraction	Saponification number.	Iodin number.	Ethyl oleate.
	° C.	Gm.	Mgm.		Per cent.
1.....	± 70 to 80	.....	<sup>a</sup> 1,397.089	.....	.....
2.....	80 to 148	5.5029	392.083	3.112	3.806
3.....	148 to 210	5.7301	350.465	6.245	7.637
4.....	210 to 248	4.1855	273.351	11.059	14.257
5.....	248 to 283	13.0994	238.216	15.290	18.697
6.....	283 to 318	54.5059	214.841	18.830	23.026
7.....	318 to 332	110.1808	198.452	25.566	31.263
Total .....	.....	193.2046	.....	.....	.....

SAMANTHA IV

1.....	± 70 to 80	.....	<sup>a</sup> 1,383.062	.....	.....
2.....	80 to 139	5.4437	397.596	2.984	3.649
3.....	139 to 195	5.6695	351.131	6.143	7.512
4.....	195 to 235	4.3474	269.935	11.561	14.137
5.....	235 to 275	13.3353	236.493	14.739	18.023
6.....	275 to 315	54.8424	213.021	18.383	22.480
7.....	315 to 329	108.1717	198.017	25.176	30.786
Total.....	.....	191.8100	.....	.....	.....

CECILE II

1.....	± 70 to 80	.....	<sup>a</sup> 1,377.451	.....	.....
2.....	80 to 153	5.4392	390.056	2.688	3.287
3.....	153 to 218	5.8774	340.175	4.798	5.867
4.....	218 to 257	4.4112	273.495	8.220	10.052
5.....	257 to 287	13.3860	240.218	10.728	13.119
6.....	287 to 317	54.8585	216.355	13.844	16.929
7.....	317 to 336	131.1700	198.795	20.386	24.929
Total.....	.....	215.1423	.....	.....	.....

<sup>a</sup> Total alkali-consuming power of the fraction.

TABLE XVI.—Weight and analysis of fractions of milk from cows intermediate in lactation (ethyl esters)—Continued

## FANCY III

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number	Iodin number.	Ethyl oleate.
	° C	Gm	Mgm.		Per cent
1.....	±70 to 80	.....	<sup>a</sup> 1,500.889	.....	.....
2.....	80 to 132.5	5.3816	401.690	1.797	2.197
3.....	132.5 to 180	5.6924	364.612	3.901	4.770
4.....	180 to 216	4.0433	291.181	8.069	9.867
5.....	216 to 264	12.9128	245.121	12.093	14.788
6.....	264 to 308	54.4026	216.675	15.754	19.265
7.....	308 to 327	109.9412	200.142	21.291	26.035
Total.....	.....	192.3739	.....	.....	.....

<sup>a</sup> Total alkali-consuming power of the fraction.

TABLE XVII.—Fatty acids in butter fat from cows intermediate in lactation

Fatty acids.	Colantha.	Samantha IV.	Cecile II.	Fancy III.
	Per cent.	Per cent.	Per cent.	Per cent.
<b>Soluble acids:</b>				
Butyric acid (by difference).....	2.525	2.777	2.492	3.028
Caproic acid.....	2.118	2.029	1.862	2.253
Caprylic acid.....	.763	.660	1.041	.881
Capric acid.....	1.338	1.237	1.378	1.380
Total.....	6.744	6.703	6.773	7.542
<b>Insoluble acids:</b>				
Lauric acid.....	5.409	4.534	5.403	5.272
Myristic acid.....	19.188	19.624	21.287	20.763
Palmitic acid (by difference).....	13.555	13.681	16.196	15.469
Stearic acid.....	14.910	15.633	18.044	15.948
Oleic acid.....	35.040	34.693	27.122	29.756
Total.....	88.102	88.165	88.052	87.208
Total fatty acids.....	94.846	94.868	94.825	94.750

The percentage of butyric acid was 2.651 in the Holsteins and 2.760 in the Jerseys; of caproic acid, 2.074 and 2.058; of caprylic acid, 0.712 and 0.961; and of capric acid, 1.288 and 1.379. In the main the results confirmed those of the previous period as to breed differences.

The percentage of lauric acid was 4.972 in the Holsteins and 5.338 in the Jerseys; of myristic acid, 19.406 and 21.025; of palmitic acid, 13.618 and 15.833; of stearic acid, 15.272 and 16.996; and of oleic acid, 34.867 and 28.439. The breed differences as to myristic and oleic acids were more decisive than in the previous period.

In the Holsteins all the soluble acids decreased in the second period, butyric most of all, and nearly all the insolubles increased, particularly oleic acid, but the agreement with the herd sample (Table II) was not materially improved. In the Jerseys the butyric and caproic acids decreased, and several of the insolubles increased, but stearic and oleic acids decreased, as a whole substantially conforming to the herd sample.

TABLE XVIII.—Weight and analysis of fractions of milk from cows late in lactation (ethyl esters)

## COLANTHA

Fraction No.	Range of fraction.	Weight of fraction.	Saponification No.	Iodin No.	Ethyl oleate.
	° C.	Gm.	Mgm.		Per cent.
1.....	± 70 to 90	.....	a <sub>1</sub> , 366.230	.....	.....
2.....	90 to 146	4.6359	400.958	6.415	7.845
3.....	146 to 211	4.8609	355.525	10.366	12.676
4.....	211 to 243	4.1081	282.435	14.466	17.689
5.....	243 to 283	14.2259	244.859	15.928	19.477
6.....	283 to 310.5	52.3540	220.233	18.799	22.988
7.....	310.5 to 331.5	67.4810	202.637	23.845	29.158
Total..	.....	147.6658	.....	.....	.....

## SAMANTHA IV

1.....	± 70 to 90	.....	a <sub>1</sub> , 371.841	.....	.....
2.....	90 to 140	4.6752	404.831	4.620	5.650
3.....	140 to 207	4.8510	357.845	8.077	9.877
4.....	207 to 244	3.9896	285.900	12.249	14.979
5.....	244 to 280	14.3177	243.531	14.524	17.761
6.....	280 to 311	52.8224	218.164	17.529	21.435
7.....	311 to 326	71.0477	202.061	22.429	27.427
Total...	.....	151.7036	.....	.....	.....

## CECILE II

1.....	± 70 to 90	.....	a <sub>1</sub> , 357.814	.....	.....
2.....	90 to 147	5.0608	406.357	5.070	6.200
3.....	147 to 191	5.3511	351.782	7.401	9.050
4.....	191 to 231	4.9009	282.018	10.028	12.263
5.....	231 to 277	14.3388	245.102	11.208	13.706
6.....	277 to 304	52.3918	220.609	13.141	16.069
7.....	304 to 321	86.4479	202.460	18.036	22.055
Total.....	.....	168.4913	.....	.....	.....

1.....	± 70 to 80	.....	a <sub>1</sub> , 402.700	.....	.....
2.....	80 to 143	4.7724	390.725	4.607	5.634
3.....	143 to 199	5.3025	369.784	6.796	8.311
4.....	199 to 235	4.1495	289.912	10.379	12.692
5.....	235 to 279	14.2308	244.922	12.682	15.508
6.....	279 to 310	53.1995	218.487	15.436	18.876
7.....	310 to 327	89.7879	200.926	20.772	25.401
Total.....	.....	171.5026	.....	.....	.....

\* Total alkali-consuming power of the fraction.

TABLE XIX.—Fatty acids in butter fat from cows late in lactation

Fatty acids.	Colantha.	Samantha IV.	Cecile II.	Fancy III.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Soluble acids:				
Butyric acid (by difference) . . . . .	2.241	2.404	2.864	3.059
Caproic acid . . . . .	1.765	1.784	1.726	2.301
Capyric acid . . . . .	.653	.733	.857	.616
Capric acid . . . . .	1.850	1.566	1.785	1.550
Total . . . . .	6.509	6.577	7.232	7.526
Insoluble acids:				
Lauric acid . . . . .	7.687	6.592	7.087	6.333
Myristic acid . . . . .	15.554	17.059	18.985	18.484
Palmitic acid (by difference) . . . . .	5.782	9.274	13.142	10.923
Stearic acid . . . . .	19.135	18.029	18.430	18.781
Oleic acid . . . . .	40.313	37.430	29.980	32.783
Total . . . . .	88.471	88.384	87.624	87.304
Total fatty acids . . . . .	94.980	94.961	94.856	94.830

The percentage of butyric acid was 2.368 in the Holsteins and 2.962 in the Jerseys; of caproic acid, 1.775 and 2.014; of caprylic acid, 0.693 and 0.737; and of capric acid, 1.708 and 1.668. The breed differences are concealed by the higher content of soluble acids in the Jerseys.

The percentage of lauric acid was 7.140 in the Holsteins and 6.710 in the Jerseys; of myristic acid, 16.307 and 18.735; of palmitic acid, 7.528 and 12.033; of stearic acid, 18.582 and 18.606; and of oleic acid, 38.872 and 31.382. The breed differences were substantially of the same character as in the intermediate period.

The Holsteins differed materially from the herd sample (Table II), particularly in the insoluble acid mixture. In a general way the Jerseys agreed with the herd sample except in stearic acid content.

#### EFFECT OF LACTATION

In the Holsteins the percentage of total fatty acids increased 0.065 in four months and 0.179 in seven months, and their neutralization number decreased 3.673 and 8.488 mgm.; the soluble fatty acids decreased 1.114 and 1.295 per cent; the insoluble fatty acids increased 1.180 and 1.474, and their neutralization number decreased 2.430 and 5.607 mgm.; and the glycerol decreased 0.159 and 0.434 per cent. The change in soluble fatty acids consisted of a decreasing loss from butyric acid to caprylic with a slight increase in capric acid. There was an appreciable gain in lauric acid, loss in myristic acid, and marked gain in oleic acid.

In the Jerseys the percentage of total fatty acids increased 0.018 in four months and 0.073 in seven months, and their neutralization number decreased 1.049 and 3.194 mgm.; the soluble fatty acids decreased 0.834 and 0.613 per cent; the insoluble fatty acids increased 0.852 and 0.682 per cent, and their neutralization number decreased 0.267 and 2.409 mgm.; and the glycerol decreased 0.043 and 0.178 per cent. The change in soluble fatty acids was more gradual than in the Holsteins; butyric, caproic, and caprylic acids decreased slightly; capric acid increased; lauric acid increased noticeably; myristic acid decreased; and oleic acid increased, but less than in the Holsteins.

#### 4. FROM MIXED MILK OF GRADE HOLSTEINS FED A NORMAL RATION, WITH AND WITHOUT THE ADDITION OF VARIOUS OILS AND FATS

Four grade Holsteins of similar age, comparatively fresh in lactation, were divided into two groups, known as herds A and B. All of the cows, except Samantha III, had been used in previous experiments. The same hay and grain mixture was fed throughout the experiment to both herds, but Colantha was reduced 2 pounds of grain in the last period. Herd B received, in addition, various oils and fats in four periods. The hay and various grains in the mixture were of fair average quality, as shown by analysis (Table XXI). The coconut fat was a refined product said to be used in the manufacture of confectionery, and the peanut and corn oils were refined for salad purposes. The soybean oil was the ordinary commercial product. They were all analyzed (Table XXII) by the common methods but were not esterified.

The milk produced was the daily average for the three days on which cream was saved for churning. The milk analyzed was a five-days' composite taken at substantially the same time as the cream sample.

TABLE XX.—Records of cows and milk analysis

	Herd A.		Herd B.	
	Colantha.	Samantha IV.	Colantha II.	Samantha III.
Breed.....	Grade Holstein....	Grade Holstein....	Grade Holstein....	Grade Holstein
Date of birth.....	Nov. 16, 1913.....	Aug. 25, 1914.....	Oct. 14, 1914.....	Aug. 25, 1913
Last calf dropped.....	Sept. 19, 1919.....	Aug. 20, 1919.....	Aug. 8, 1919.....	Sept. 12, 1919.
Condition on calving .....	Good flesh.....	Good flesh.....	Good flesh.....	Good flesh.
Date served.....	Dec. 5, 1919; Feb. 4, Mar. 14, 1920.	Dec. 4, 1919.....	Nov. 3, 1919.....	Dec. 8, 1919.

PERIOD I				
Sample No.....	A1.....	A1.....	B1.....	B1.....
Daily ration:				
Hay.....	24 pounds.....	24 pounds.....	22 pounds.....	24 pounds.
Grain mixture.....	10 pounds.....	10 pounds.....	10 pounds.....	10 pounds.
Gluten feed, 40 per cent.....				
Wheat bran, 20 per cent.....				
Barley meal, 40 per cent.....				
Weight of animal.....	1,348 pounds.....	1,160 pounds.....	1,093 pounds.....	1,210 pounds.
Milk produced (daily average). <sup>1</sup>	Nov. 18 to 20, 1919, 29.6 pounds.	Nov. 18 to 20, 1919, 34.7 pounds.	Nov. 18 to 20, 1919, 31.4 pounds	Nov. 18 to 20, 1919, 29.2 pounds.
Milk analysis:				
Solids (gravimetric).....	12.66 per cent.....	12.84 per cent.....	13.29 per cent.....	13.25 per cent.
Fat (Babcock).....	4.23 per cent.....	4.50 per cent.....	4.60 per cent.....	4.58 per cent
Proteids (N X 6.25).....	3.27 per cent.....	3.05 per cent.....	3.29 per cent.....	3.48 per cent
Lactose (by difference).....	4.39 per cent.....	4.60 per cent.....	4.60 per cent.....	4.44 per cent.
Ash.....	.77 per cent.....	.69 per cent.....	.71 per cent.....	.75 per cent.
Sample No.....	A2.....	A2.....	B2.....	B2.....
Weight of animal.....	1,308 pounds.....	1,125 pounds.....	1,083 pounds.....	1,183 pounds.
Milk produced (daily average). <sup>1</sup>	Nov. 27 to 29, 1919, 24.6 pounds.	Nov. 27 to 29, 1919, 31.6 pounds.	Nov. 27 to 29, 1919, 31.0 pounds.	Nov. 27 to 29, 1919, 25.2 pounds.
Milk analysis:				
Solids (gravimetric).....	12.65 per cent.....	12.91 per cent.....	13.10 per cent.....	13.35 per cent.
Fat (Babcock).....	4.43 per cent.....	4.59 per cent.....	4.38 per cent.....	4.48 per cent
Proteids (N X 6.25).....	3.43 per cent.....	3.09 per cent.....	3.35 per cent.....	3.58 per cent.
Lactose (by difference).....	4.00 per cent.....	4.63 per cent.....	4.64 per cent.....	4.54 per cent.
Ash.....	0.79 per cent.....	0.69 per cent.....	0.73 per cent.....	0.75 per cent.

For the days cream was saved for churning.

TABLE XX.—Records of cows and milk analysis—Continued

## PERIOD 2

	Herd A.		Herd B.	
	Colantha.	Samantha IV.	Colantha II.	Samantha III.
Sample No. ....	A3.....	A3.....	B3.....	B1.
Daily ration:				
Hay.....	24 pounds.....	24 pounds.....	22 pounds.....	24 pounds.....
Grain mixture.....	10 pounds.....	10 pounds.....	10 pounds.....	10 pounds.....
Coconut fat.....			.75 pounds.....	.75 pounds.....
Weight of animal.....	1,298 pounds.....	1,183 pounds.....	1,108 pounds.....	1,213 pounds.....
Milk produced (daily average). <sup>1</sup>	Dec. 11 to 11, 1919, 18.2 pounds.	Dec. 11 to 13, 1919, 31.5 pounds.	Dec. 11 to 11, 1919, 58.5 pounds.	Dec. 11 to 11, 1919, 25.6 pounds.
Milk analysis:				
Solids (gravimetric)....	12.66 per cent.....	13.04 per cent.....	13.81 per cent.....	14.12 per cent.....
Fat (Babcock).....	4.10 per cent.....	4.60 per cent.....	5.30 per cent.....	5.35 per cent.....
Proteids (N X 6.25)....	3.47 per cent.....	3.09 per cent.....	3.39 per cent.....	3.46 per cent.....
Lactose (by difference)...	4.29 per cent.....	4.65 per cent.....	4.59 per cent.....	4.54 per cent.....
Ash.....	.80 per cent.....	.70 per cent.....	.73 per cent.....	.77 per cent.....
Sample No. ....	A4.....	A4.....	B4.....	B4.
Weight of animal.....	1,333 pounds.....	1,203 pounds.....	1,123 pounds.....	1,255 pounds.....
Milk produced (daily average). <sup>1</sup>	Dec. 25 to 27, 1919, 23.7 pounds.	Dec. 25 to 27, 1919, 31.6 pounds.	Dec. 25 to 27, 1919, 24.3 pounds.	Dec. 25 to 27, 1919, 24.6 pounds.
Milk analysis:				
Solids (gravimetric)....	12.96 per cent.....	12.87 per cent.....	13.31 per cent.....	14.20 per cent.....
Fat (Babcock).....	4.38 per cent.....	4.40 per cent.....	5.20 per cent.....	5.60 per cent.....
Proteids (N X 6.25)....	3.44 per cent.....	3.09 per cent.....	3.05 per cent.....	3.44 per cent.....
Lactose (by difference)...	4.35 per cent.....	4.68 per cent.....	4.35 per cent.....	4.40 per cent.....
Ash.....	.79 per cent.....	.70 per cent.....	.71 per cent.....	.76 per cent.....

## PERIOD 3

Sample No. ....	A5.....	A5.....	B5.....	B5.
Daily ration:				
Hay.....	24 pounds.....	24 pounds.....	22 pounds.....	24 pounds.....
Grain mixture.....	10 pounds.....	10 pounds.....	10 pounds.....	10 pounds.....
Peanut oil.....			.75 pounds.....	.75 pounds.....
Weight of animal.....	1,315 pounds.....	1,178 pounds.....	1,080 pounds.....	1,230 pounds.....
Milk produced (daily average). <sup>1</sup>	Jan. 8 to 10, 1920, 21.8 pounds.	Jan. 8 to 10, 1920, 30.1 pounds.	Jan. 8 to 10, 1920, 33.9 pounds.	Jan. 8 to 10, 1920, 27.7 pounds.
Milk analysis:				
Solids (gravimetric)....	12.69 per cent.....	13.06 per cent.....	12.92 per cent.....	13.19 per cent.....
Fat (Babcock).....	4.15 per cent.....	4.60 per cent.....	4.20 per cent.....	4.78 per cent.....
Proteids (N X 6.25)....	3.47 per cent.....	3.12 per cent.....	3.22 per cent.....	3.20 per cent.....
Lactose (by difference)...	4.29 per cent.....	4.64 per cent.....	4.77 per cent.....	4.57 per cent.....
Ash.....	.78 per cent.....	.70 per cent.....	.71 per cent.....	.75 per cent.....
Sample No. ....	A6.....	A6.....	B6.....	B6.
Weight of animal.....	1,338 pounds.....	1,200 pounds.....	1,080 pounds.....	1,223 pounds.....
Milk produced (daily average). <sup>1</sup>	Jan. 22 to 24, 1920, 20.8 pounds.	Jan. 22 to 24, 1920, 31.8 pounds.	Jan. 22 to 24, 1920, 33.1 pounds.	Jan. 22 to 24, 1920, 29.9 pounds.
Milk analysis:				
Solid (gravimetric)....	13.09 per cent.....	13.13 per cent.....	13.17 per cent.....	13.14 per cent.....
Fat (Babcock).....	4.45 per cent.....	4.65 per cent.....	4.15 per cent.....	4.40 per cent.....
Proteids (N X 6.25)....	3.51 per cent.....	3.17 per cent.....	3.11 per cent.....	3.14 per cent.....
Lactose (by difference)...	4.37 per cent.....	4.62 per cent.....	4.80 per cent.....	4.67 per cent.....
Ash.....	.76 per cent.....	.69 per cent.....	.71 per cent.....	.73 per cent.....

## PERIOD 4

Sample No. ....	A7.....	A7.....	B7.....	B7.
Daily ration:				
Hay.....	24 pounds.....	24 pounds.....	22 pounds.....	24 pounds.....
Grain mixture.....	10 pounds.....	10 pounds.....	10 pounds.....	10 pounds.....
Corn oil.....			.75 pound.....	.75 pound.....
Weight of animal.....	1,348 pounds.....	1,182 pounds.....	1,084 pounds.....	1,231 pounds.....
Milk produced (daily average). <sup>1</sup>	Feb. 5 to 7, 1920, 22 pounds.	Feb. 5 to 7, 1920, 29.6 pounds.	Feb. 5 to 7, 1920, 35.4 pounds.	Feb. 5 to 7, 1920, 31 pounds.
Milk analysis:				
Solids (gravimetric)....	13.27 per cent.....	13.11 per cent.....	12.58 per cent.....	12.92 per cent.....
Fat (Babcock).....	4.45 per cent.....	4.65 per cent.....	4 per cent.....	4.33 per cent.....
Proteids (N X 6.25)....	3.58 per cent.....	3.16 per cent.....	3.14 per cent.....	3.26 per cent.....
Lactose (by difference)...	4.77 per cent.....	4.61 per cent.....	4.74 per cent.....	4.61 per cent.....
Ash.....	.77 per cent.....	.69 per cent.....	.70 per cent.....	.72 per cent.....
Sample No. ....	A8.....	A8.....	B8.....	B8.
Weight of animal.....	1,343 pounds.....	1,168 pounds.....	1,085 pounds.....	1,218 pounds.....

<sup>1</sup> For the days cream was saved for churning

TABLE XX.—Records of cows and milk analysis—Continued

## PERIOD 4—Continued

	Herd A.		Herd B.	
	Colantha.	Samantha IV.	Colantha II.	Samantha III.
Milk produced (daily average). <sup>1</sup>	Feb. 19 to 27, 1920, 20.6 pounds	Feb. 19 to 27, 1920, 31.3 pounds.	Feb. 19 to 27, 1920, 33.7 pounds.	Feb. 19 to 27, 1920, 29 pounds.
Milk analysis:				
Solids (gravimetric)....	13.06 per cent.....	12.96 per cent.....	12.47 per cent.....	12.63 per cent.
Fat (Babcock).....	4.40 per cent.....	4.50 per cent.....	4.00 per cent.....	4.20 per cent.
Proteids (N×6.25).....	3.45 per cent.....	3.14 per cent.....	3.09 per cent.....	3.08 per cent.
Lactose (by difference).....	4.45 per cent.....	4.61 per cent.....	4.66 per cent.....	4.61 per cent.
Ash.....	.76 per cent.....	.71 per cent.....	.72 per cent.....	.74 per cent.

## PERIOD 5

	As.....	As.....	Bs.....	Bs.....
Sample No.....				
Daily ration.....				
Hay.....	24 pounds.....	24 pounds.....	22 pounds.....	24 pounds.
Grain mixture.....	8 pounds.....	10 pounds.....	10 pounds.....	10 pounds.
Soybean oil.....			.75 pounds.....	.75 pounds.
Weight of animal.....	1,353 pounds.....	1,193 pounds.....	1,098 pounds.....	1,220 pounds.
Milk produced (daily average). <sup>1</sup>	Mar. 4 to 6, 1920, 21.6 pounds	Mar. 4 to 6, 1920, 29.8 pounds.	Mar. 4 to 6, 1920, 31.8 pounds.	Mar. 6 to 8, 1920, 29.1 pounds.
Milk analysis:				
Solids (gravimetric)....	12.75 per cent.....	13.16 per cent.....	12.99 per cent.....	12.83 per cent.
Fat (Babcock).....	4.25 per cent.....	4.60 per cent.....	4.20 per cent.....	4 per cent.
Proteids (N×6.25).....	3.37 per cent.....	3.17 per cent.....	3.22 per cent.....	3.18 per cent.
Lactose (by difference).....	4.38 per cent.....	4.69 per cent.....	4.82 per cent.....	4.91 per cent.
Ash.....	.75 per cent.....	.70 per cent.....	.73 per cent.....	.74 per cent.
Sample No.....	As.....	As.....	Bs.....	Bs.....
Weight of animal.....	1,345 pounds.....	1,170 pounds.....	1,088 pounds.....	1,245 pounds.
Milk produced (daily average). <sup>1</sup>	Mar. 18 to 20, 1920, 22.3 pounds.	Mar. 18 to 20, 1920, 30.1 pounds.	Mar. 18 to 20, 1920, 27.3 pounds.	Mar. 18 to 20, 1920, 27.5 pounds.
Milk analysis:				
Solids (gravimetric)....	12.95 per cent.....	13.30 per cent.....	13.79 per cent.....	12.91 per cent.
Fat (Babcock).....	4.30 per cent.....	4.70 per cent.....	4.49 per cent.....	4.20 per cent.
Proteids (N×6.25).....	3.37 per cent.....	3.45 per cent.....	3.61 per cent.....	3.79 per cent.
Lactose (by difference).....	4.55 per cent.....	4.77 per cent.....	5.09 per cent.....	4.80 per cent.
Ash.....	.73 per cent.....	.68 per cent.....	.76 per cent.....	.72 per cent.

<sup>1</sup> For the days cream was saved for churning.

TABLE XXI.—Analysis of feeds

	Hay.	Gluten feed.	Wheat bran.	Barley meal.
	Per cent.	Per cent.	Per cent.	Per cent.
Moisture.....	10.59	10.20	12.19	12.77
Dry matter:				
Ash.....	6.03	4.52	7.31	2.45
Protein (N×6.25).....	8.73	27.94	16.75	12.32
Fiber.....	32.38	6.32	11.29	4.86
Extract matter.....	50.55	58.43	59.74	77.91
Fat.....	2.31	2.79	4.91	2.46

TABLE XXII.—Analysis of oils and fats fed

	Coconut fat.	Peanut oil	Corn oil.	Soybean oil.
Saponification number ( <i>s</i> ).....(mgm.)..	256.600	189.835	190.761	192.337
Acid number ( <i>a</i> ).....(mgm.)..	.092	.532	.347	2.594
Ether number ( <i>e</i> ).....(mgm.)..	256.508	189.303	190.414	189.743
Iodin number.....	9.447	94.751	123.319	131.180
Equivalent in oleic acid....(per cent)...	10.508	.....	.....	.....
Total fatty acids ( <i>T</i> ) (1.00—0.00022594 <i>e</i> )	.....	.....	.....	.....
.....(per cent)...	94.204	95.723	95.698	95.713
Neutralization number( <i>n</i> )/ <i>T</i> (mgm.)..	272.388	198.317	199.336	200.952
Free fatty acids ( <i>A</i> ) <i>a/n</i> .....(per cent)...	.034	.268	.174	1.291
Soluble fatty acids ( <i>S</i> ) ( <i>T</i> — <i>I</i> ) (per cent)...	9.372	None.	None.	None.
Neutralization number.....(mgm.)..	341.539	.....	.....	.....
Insoluble fatty acids ( <i>I</i> ) by alcoholic potash.....(per cent)...	84.832	96.005	96.026	95.953
Neutralization number.....(mgm.)..	264.748	<sup>a</sup> 211.577	<sup>a</sup> 219.463	<sup>a</sup> 219.425
Stearic acid by crystallization.....(per cent)...	2.755	None.	.523	.334
Glycerol (0.00054703 <i>e</i> ).....(per cent)...	14.032	10.355	10.416	10.380
Refractive index (Abbe) 25° C ..	1.4550	1.4689	1.4724	1.4737
Refractive index (Abbe) 40° C.....	1.4491	1.4632	1.4671	1.4677
Colorimeter (Lovibond) ½-inch cell {	<i>b</i> .57	<i>b</i> 1.05	<i>b</i> 2.70	<i>b</i> 16.10
	<i>c</i> .03	<i>c</i> .45	<i>c</i> .30	<i>c</i> 3.90
Viscosity (Redwood) 70° F.....	8.691	11.554	10.203	9.730

<sup>a</sup> Exceeds the neutralization number of the total fatty acids due to decomposition difficult to control in such unsaturated products.

*b* Yellow.

*c* Orange.

The composition of the milk (Table XX) produced by the control herd A on hay and grain was fairly constant during the several periods of the experiment (four months), as was also the yield except in the case of Colantha in the second period when there was a noticeable decrease due to a sore teat. The average analysis of the milk of herd A for all periods and that of herd B for each period, together with the yields, are presented in Table XXIII.

TABLE XXIII.—Summarized milk data

	Herd A.	Herd B.				
	Periods 1 to 5.	Period 1, preliminary.	Period 2, coconut fat.	Period 3, peanut oil.	Period 4, corn oil.	Period 5, soybean oil.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Solids .....	12.96	13.25	13.86	13.16	12.65	13.13
Fat.....	4.44	4.51	5.34	4.43	4.14	4.20
Proteids.....	3.28	3.43	3.31	3.29	3.14	3.30
Lactose.....	4.51	4.57	4.47	4.71	4.65	4.89
Ash.....	.73	.74	.74	.73	.72	.74
Average production for the period .....	<i>Pounds.</i> 27.3	<i>Pounds.</i> 29.7	<i>Pounds.</i> 25.2	<i>Pounds.</i> 31.5	<i>Pounds.</i> 32.4	<i>Pounds.</i> 29.7



There was a substantial agreement in composition of the milk of herd A and that of herd B in the preliminary period, and the yield was similar. It is logical to assume that the composition of the milk of herd B in subsequent periods would have remained fully as constant as that of herd A provided the oils and fats fed were without influence. As compared with the preliminary period, coconut fat increased the solids 0.61 per cent and the fat 0.83 per cent, but at a loss of 4.5 pounds of milk a day for the period. On peanut oil the milk practically reverted to its original composition, with a gain in yield of 1.8 pounds daily over the preliminary period.

By a like comparison, corn oil decreased the solids 0.60 per cent and the fat 0.37 per cent but with an increased yield of 2.7 pounds of milk, and on soybean oil the solids nearly equaled those of the preliminary period, with a loss of 0.31 per cent of fat and approximately the same yield.

#### ANALYSIS OF BUTTER FAT FROM HERD A

Colantha and Samantha IV were considered as herd A. The cream from these two cows was mixed and churned, and samples of butter fat were analyzed; but as the maximum range did not exceed a reasonable variation for such a physiological product over a period of four months, three samples, A<sub>2</sub>, A<sub>6</sub>, and A<sub>10</sub> (averaging practically the same as the 10 samples) were deemed sufficient to fully represent the herd and for subsequent esterification.

The percentages of total fatty acids in A<sub>2</sub>, A<sub>6</sub>, and A<sub>10</sub> were 94.771, 94.806, and 94.802 (average 94.793) with no appreciable variation, and their neutralization numbers were 246.212, 244.913, and 243.878 mgm. (average 245.001), with a loss of 2.334 mgm., largely due in advancing lactation to a slight increase of insoluble acids at the expense of the soluble. The percentages of free fatty acids were 0.772, 0.936, and 0.458 (average 0.722), indicating a moderate amount of hydrolysis largely due to manipulation. The percentages of soluble fatty acids were 7.591, 7.302, and 7.258 (average 7.384), and their neutralization numbers 496.614, 491.413, and 498.044 mgm. (average 495.357).

The percentages of insoluble fatty acids were 87.180, 87.504, and 87.544 (average 87.409), and their neutralization numbers 224.409, 224.343, and 222.806 mgm. (average 223.853), indicating a slight increase in the proportion of high molecular weight acids with advancing lactation. The percentages of glycerol were 12.660, 12.576, and 12.586 (average 12.607), indicating no marked change.

As compared with the herd sample (Table I) the average of A<sub>2</sub>, A<sub>6</sub>, and A<sub>10</sub> showed a close agreement in percentage of total, soluble, and insoluble fatty acids and glycerol; but the neutralization number of the total fatty acids was higher by 0.901 mgm., that of the soluble acids was lower by 14.262 mgm., and that of the insoluble acids was higher by 1.963 mgm., indicating some difference in proportion of constituent acids.

TABLE XXIV.—Analysis of butter fat

Sample No.	Period 1.		Period 2.		Period 3.		Period 4.		Period 5.	
	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	A <sub>5</sub>	A <sub>6</sub>	A <sub>7</sub>	A <sub>8</sub>	A <sub>9</sub>	A <sub>10</sub>
Saponification number (s).....	233.179	233.338	236.973	232.471	232.986	232.192	231.213	231.838	231.171	231.201
Acid number (a).....	1.661	1.900	1.787	1.923	1.972	2.262	1.474	1.831	1.238	1.118
Ether number (e).....	231.518	231.438	236.186	230.548	231.014	239.900	229.739	229.967	229.943	230.083
Iodine number (i).....	27.312	28.017	28.831	27.361	27.631	27.714	27.593	28.253	28.294	28.649
Free fatty acids (A).....	30.381	31.165	32.070	30.435	30.736	30.828	31.027	31.428	31.473	31.868
Total fatty acids (T) (see 3394 e).....	94.769	94.771	94.822	94.791	94.760	94.866	94.869	94.864	94.865	94.862
Neutralization number (n) (1/T).....	246.930	246.212	245.588	245.246	245.818	244.913	243.872	244.523	243.838	243.878
Free fatty acids (A) %.....	7.952	7.772	7.764	7.764	7.862	7.916	7.664	7.757	7.594	7.458
Soluble fatty acids (S) (T - i).....	462.997	465.014	465.664	465.616	465.310	468.413	461.330	468.112	468.378	468.044
Insoluble fatty acids (I) by alcoholic potash.....	86.783	87.180	87.376	87.391	87.364	87.364	87.330	87.268	87.517	87.544
Neutralization number.....	216.089	214.409	214.349	214.263	214.843	214.364	213.368	213.368	213.474	212.866
Steric acid by crystallization.....	9.967	10.345	8.288	5.544	9.101	9.900	12.141	12.586	12.570	12.586
Glycerol (0.0095703 e).....	12.665	12.660	12.337	12.612	12.537	12.576	12.597	12.586	12.570	12.586
Refractive index (Abbe) 40° C.....	1.4536	1.4536	1.4536	1.4536	1.4536	1.4536	1.4536	1.4536	1.4535	1.4534
Colorimeter (Lovibond) 1/2-inch cell.....	3.20	3.70	3.30	3.00	3.20	2.80	2.00	2.10	2.10	2.20
	1.80	1.70	1.50	1.30	1.00	1.00	1.10	1.10	1.10	1.20

e Yellow.

b Orange.

TABLE XXV.—Weight and analysis of fractions (ethyl esters)

A <sub>2</sub>					
Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1.....			<sup>a</sup> 1,585.051		
2.....	90 to 144.5	4.7939	401.384	2.593	3.171
3.....	144.5 to 199	5.4274	347.415	6.021	7.362
4.....	199 to 238	5.1062	272.158	11.306	13.825
5.....	238 to 278.5	15.7266	238.796	14.195	17.358
6.....	278.5 to 310	54.5945	215.353	16.903	20.669
7.....	310 to 325	109.4460	199.769	22.105	27.031
Total.....		195.0946			

A <sub>8</sub>					
1.....	±70 to 90		<sup>a</sup> 1,419.532		
2.....	90 to 148	4.7288	398.816	2.991	3.658
3.....	148 to 199.5	5.1199	356.153	5.810	7.105
4.....	199.5 to 238.5	4.8099	281.241	10.578	12.935
5.....	238.5 to 281	15.7429	240.099	13.946	17.054
6.....	281 to 312.5	53.7111	215.163	16.509	20.187
7.....	312.5 to 328	125.3339	198.528	22.400	27.391
Total.....		209.4465			

A <sub>10</sub>					
1.....	±70 to 90		<sup>a</sup> 1,447.586		
2.....	90 to 149.5	4.6372	396.237	2.414	2.951
3.....	149.5 to 214	5.2042	363.557	5.083	6.216
4.....	214 to 258.5	4.9114	274.142	11.359	13.891
5.....	258.5 to 294.5	15.6960	235.719	14.710	17.088
6.....	294.5 to 318.5	54.0467	212.990	17.636	21.566
7.....	318.5 to 337	128.5638	197.798	24.760	30.277
Total.....		213.0593			

<sup>a</sup> Total alkali-consuming power of the fraction

TABLE XXVI.—Fatty acids in butter fat

Fatty acids.	A <sub>2</sub>	A <sub>4</sub>	A <sub>10</sub>
Soluble acids:	Per cent.	Per cent.	Per cent.
Butyric acid (by difference).....	3.407	3.120	3.480
Caproic acid.....	1.731	1.872	2.064
Caprylic acid.....	.810	.820	.527
Capric acid.....	1.643	1.490	1.187
Total.....	7.591	7.302	7.258
Insoluble acids:			
Lauric acid.....	5.806	5.415	4.913
Myristic acid.....	20.682	20.297	20.931
Palmitic acid (by difference).....	20.508	22.257	19.103
Stearic acid.....	9.019	8.707	10.729
Oleic acid.....	31.165	30.828	31.868
Total.....	87.180	87.504	87.544
Total fatty acids.....	94.771	94.806	94.802

The percentages of butyric acid in  $A_2$ ,  $A_8$ , and  $A_{10}$ , from cows receiving grain and hay, were 3.407, 3.120, and 3.480 (average 3.336); of caproic acid 1.731, 1.872, and 2.064 (average 1.889); of caprylic acid 0.810, 0.820, and 0.527 (average 0.719); and of capric acid 1.643, 1.490, and 1.187 (average 1.440). The butyric and caproic acids fully maintained their percentage during the four months of the experiment, but the caprylic and capric acids decreased.

As compared with the herd sample (Table II) the butyric acid averaged higher by 0.183 per cent, the caproic acid higher by 0.529 per cent, the caprylic acid lower by 0.256 per cent, and the capric acid lower by 0.391 per cent.

The percentages of lauric acid were 5.806, 5.415, and 4.913 (average 5.378); of the myristic acid 20.682, 20.297, and 20.931 (average 20.637); of the palmitic acid 20.508, 22.257, and 19.103 (average 20.623); of the stearic acid 9.019, 8.707, and 10.729 (average 9.485); and of the oleic acid 31.165, 30.828, and 31.868 (average 31.287). The lauric acid decreased with advancing lactation; otherwise there was no consistent change noted.

As compared with the herd sample (Table II) the lauric acid was low by 1.517 per cent, the myristic acid low by 1.981 per cent, the palmitic acid high by 5.165 per cent, the stearic acid low by 1.899 per cent, and the oleic acid high by 0.142 per cent, a noticeable difference in proportion of constituent acids.

#### ANALYSIS OF BUTTER FAT FROM HERD B

Colantha II and Samantha III were considered as herd B. The cream was mixed and churned and 10 samples of butter fat, representing five feeding periods, were analyzed. The first sample in each period was considered indicative, but the second, taken after the same feeding had been continued two weeks longer, was regarded as a better criterion of the effect of the oils and fats fed and consequently for esterification.

Fat samples  $B_1$  and  $B_2$  were of similar character, differing somewhat in content of soluble fatty acids. As compared with  $A_2$ ,  $B_2$  contained practically the same amount of total, free, soluble, and insoluble fatty acids and of glycerol; but the neutralization number of the insoluble acids was slightly higher, 1.174 mgm. It again appears reasonable to assume that, if the feeding of oils and fats was without influence, the composition of the butter fat of herd B should continue fully as uniform as that from herd A, thus permitting a direct comparison of the preliminary  $B_2$  and subsequent feeding periods with possibly a slight allowance for advancing lactation.

The soluble fatty acids in  $B_1$ , from cows receiving coconut fat, decreased 1.243 per cent, as compared with  $B_2$ ; the insoluble fatty acids increased 1.249 per cent and their neutralization number 3.194 mgm., a decided change both in quantity and character of the insoluble acids.

The total fatty acids in  $B_6$ , from cows receiving peanut oil, increased 0.226 per cent, and their neutralization number decreased 11.213 mgm.; the soluble fatty acids decreased 1.206 per cent, and their neutralization number, 20.055; the insoluble fatty acids increased 1.432 per cent, and their neutralization number decreased 6.983; and the glycerol decreased 0.548 per cent, indicating a notable gain in proportion of higher molecular weight acids in both groups.

TABLE XXVII.—Analysis of butter fat

	Period 1.		Period 2.		Period 3.		Period 4.		Period 5.	
	B 1	B 2	B 3	B 4	B 1	B 2	B 1	B 2	B 1	B 2
Sample number . . . . .	232, 634	231, 788	234, 400	233, 334	226, 015	223, 605	221, 837	220, 751	222, 552	221, 622
Saponification number (s) . . . . .	1, 376	1, 866	1, 693	1, 591	1, 498	1, 786	2, 610	3, 015	1, 759	1, 948
Acid number (a) . . . . .	231, 258	231, 922	232, 707	231, 643	224, 517	221, 909	221, 227	216, 836	220, 793	219, 674
Ether number (e) . . . . .	27, 726	27, 063	25, 307	26, 511	36, 248	37, 982	38, 879	40, 423	41, 090	41, 218
Iodine number . . . . .	30, 841	30, 771	28, 150	29, 490	40, 321	42, 250	44, 360	44, 965	45, 707	45, 849
Equivalent in oleic acid . . . . .	94, 775	94, 705	94, 742	94, 706	94, 927	94, 986	95, 002	95, 101	95, 037	95, 037
Unsaturation number (n) (s) f . . . . .	245, 459	246, 710	247, 468	246, 116	238, 093	235, 303	235, 613	232, 123	234, 238	233, 195
Free fatty acids (A) (s) f . . . . .	7, 583	7, 750	7, 684	7, 646	6, 069	6, 758	1, 108	1, 067	1, 751	1, 835
Soluble fatty acids (S) (T-f) . . . . .	7, 388	7, 556	7, 479	7, 438	5, 861	6, 550	6, 730	6, 695	6, 771	6, 708
Neutralization number . . . . .	475, 329	487, 796	480, 214	481, 253	415, 961	408, 715	418, 530	418, 566	413, 991	446, 174
Insoluble fatty acids (f) by alcoholic potash (per cent) . . . . .	87, 487	87, 004	87, 466	88, 323	87, 966	88, 323	88, 323	88, 323	88, 323	88, 323
Neutralization number . . . . .	226, 310	225, 583	228, 918	228, 777	219, 269	218, 600	221, 032	218, 470	217, 458	218, 414
Stearic acid by crystallization . . . . .	10, 351	8, 966	10, 971	9, 754	14, 137	14, 716	13, 486	14, 968	14, 968	15, 277
Glycerol (0.0094793 e) . . . . .	12, 651	12, 687	12, 730	12, 672	12, 282	12, 139	12, 108	11, 862	12, 078	12, 037
Refractive index (Abbe) 40° C. . . . .	1, 4536	1, 4536	1, 4536	1, 4549	1, 4551	1, 4555	1, 4553	1, 4553	1, 4556	1, 4556
Colorimeter (Lovibond) 1/2-inch cell . . . . .	5.86	5.70	6.390	6.366	6.235	6.280	6.200	6.210	6.240	6.240
	1.70	1.86	1.50	1.25	0.95	1.10	1.40	1.20	1.20	1.20

\* Yellow.

b Orange.

The total fatty acids in  $B_9$ , from cows receiving corn oil, increased 0.341 per cent as compared with  $B_2$ , and their neutralization number decreased 14.593 mgm.; the free fatty acids increased 0.931 per cent; the soluble acids decreased 1.131 per cent, and their neutralization number, 65.274 mgm.; the insoluble acids increased 1.472 per cent, and their neutralization number decreased 7.416 mgm.; and the glycerol decreased 0.825 per cent, a change similar to but more marked than in the previous period.

The total fatty acids in  $B_{10}$ , from cows receiving soybean oil, increased 0.277 per cent, and their neutralization number decreased 13.521 mgm.; the soluble fatty acids decreased 1.588 per cent, and their neutralization number 37.606 mgm.; the insoluble fatty acids increased 1.865 per cent, and their neutralization number decreased 7.169 mgm.; and the glycerol decreased 0.670 per cent, a change slightly different but approximately the same as in the previous case.

TABLE XXVIII.—Weight and analysis of fractions (ethyl esters)

$B_2$					
Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	<sup>°C.</sup>	Gm.	Mgm		Per cent.
1.....	± 70 to 90	.....	<sup>a</sup> 1,587.856	.....	.....
2.....	90 to 153	4.7042	387.750	3.158	3.862
3.....	153 to 207	5.2264	367.367	5.541	6.776
4.....	207 to 251	4.8993	288.957	10.324	12.625
5.....	251 to 290.5	15.8992	245.752	13.679	16.727
6.....	290.5 to 316.5	53.9656	217.611	16.639	20.347
7.....	316.5 to 334.5	131.8435	199.906	22.974	28.094
Total.....	.....	216.5382	.....	.....	.....

$B_4$					
1.....	± 70 to 90	.....	<sup>a</sup> 1,441.976	.....	.....
2.....	90 to 138	4.4956	403.891	1.939	2.371
3.....	138 to 201	5.0411	366.749	4.526	5.534
4.....	201 to 245	4.8221	275.390	9.817	12.005
5.....	245 to 274.5	15.5843	239.613	12.513	15.301
6.....	274.5 to 303.5	53.5750	219.938	14.983	18.322
7.....	303.5 to 327.5	132.2908	202.036	22.110	27.037
Total.....	.....	215.8089	.....	.....	.....

$B_6$					
1.....	± 70 to 90	.....	<sup>a</sup> 1,554.192	.....	.....
2.....	90 to 138.5	4.6859	495.759	3.130	3.828
3.....	138.5 to 192	5.1400	362.188	6.403	7.830
4.....	192 to 238	4.8029	270.068	14.655	17.921
5.....	238 to 284	15.5928	231.380	19.448	23.782
6.....	284 to 314	53.7289	209.791	24.451	29.900
7.....	314 to 328	103.0110	195.994	32.311	39.511
Total.....	.....	186.9615	.....	.....	.....

<sup>a</sup> Total alkali-consuming power of the fraction..

TABLE XXVIII.—Weight and analysis of fractions—Continued

B<sub>8</sub>

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1.....	± 70 to 95	.....	a <sub>1</sub> , 453.197	.....	.....
2.....	95 to 148.5	4.4388	395.177	3.451	4.220
3.....	148.5 to 198	4.4753	359.884	6.806	8.323
4.....	198 to 246	4.6266	267.181	15.492	18.944
5.....	246 to 288	14.4314	231.499	20.229	24.737
6.....	288 to 316.5	53.7554	209.624	25.724	31.456
7.....	316.5 to 333.5	99.2487	195.484	34.729	42.468
Total.....	.....	180.9762	.....	.....	.....

B<sub>10</sub>

1.....	± 70 to 97	.....	a <sub>1</sub> , 590.662	.....	.....
2.....	97 to 142	4.3775	402.010	4.151	5.076
3.....	142 to 186	4.6831	360.950	7.348	8.985
4.....	186 to 234	4.5210	271.797	15.174	18.555
5.....	234 to 273	13.8912	231.688	20.457	25.016
6.....	273 to 317	52.8181	210.453	25.743	31.479
7.....	317 to 327.5	96.9084	195.983	34.205	41.827
Total.....	.....	177.1993	.....	.....	.....

<sup>a</sup> Total alkali-consuming power of the fraction.

TABLE XXIX.—Fatty acids in butter fat

Fatty acids,	B <sub>8</sub> preliminary.	B <sub>8</sub> coconut fat.	B <sub>8</sub> peanut oil.	B <sub>8</sub> Corn oil.	B <sub>10</sub> soybean oil.
Soluble acids:	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Butyric acid (by difference).....	2.669	2.597	3.124	3.272	2.893
Caproic acid.....	2.263	1.972	1.921	1.829	1.779
Caprylic acid.....	.816	.452	.468	.401	.474
Capric acid.....	2.008	1.492	1.037	1.123	1.022
Total.....	7.756	6.513	6.550	6.625	6.168
Insoluble acids:					
Lauric acid.....	6.210	8.042	4.681	4.551	4.839
Myristic acid.....	22.050	24.988	17.223	16.428	16.128
Palmitic acid (by difference).....	20.170	17.125	11.268	<sup>a</sup> 9.262	8.699
Stearic acid.....	7.803	8.608	13.014	13.270	13.354
Oleic acid.....	30.771	29.490	42.250	44.965	45.849
Total.....	87.004	88.253	88.436	88.476	88.869
Total fatty acids.....	94.760	94.766	94.986	95.101	95.037

<sup>a</sup> In these abnormal samples the recovery by distillation exceeded the results reported by 0.698 per cent in B<sub>8</sub> and by 0.684 in B<sub>10</sub>. It is impossible to ascribe definitely the source of this error at the present writing.

As compared with  $A_2$ ,  $B_2$  on a normal ration contained less butyric acid by 0.738 per cent, more caproic acid by 0.532 per cent, more caprylic acid by 0.006 per cent, and more capric acid by 0.365 per cent, differences too great to permit the direct use of the former as a basis.

In the case of the insoluble acids,  $B_2$  contained more lauric acid by 0.404 per cent, more myristic acid by 1.368 per cent, less palmitic acid by 0.338 per cent, less stearic acid by 1.216 per cent, and less oleic acid by 0.394 per cent—a much closer agreement than in the case of the soluble acids, considering the amounts involved, but still making it advisable to use only  $B_2$  for direct comparison with the other periods.

As compared with the herd sample (Table II) the differences are even greater.  $B_2$  contained less butyric acid by 0.484 per cent, more caproic acid by 0.903 per cent, less caprylic acid by 0.159 per cent, more capric acid by 0.177 per cent, less lauric acid by 0.685 per cent, less myristic acid by 0.568 per cent, more palmitic acid by 4.712 per cent, less stearic acid by 3.581 per cent, and less oleic acid by 0.374 per cent.

The butyric acid in  $B_4$ , from the cows receiving coconut fat, decreased 0.072 per cent as compared with  $B_2$ ; caproic acid decreased 0.291 per cent; caprylic acid 0.364 per cent; capric acid 0.516 per cent; lauric acid increased 1.832 per cent; myristic acid increased 2.938 per cent; palmitic acid decreased 3.045 per cent; stearic acid increased 0.805 per cent; and oleic acid decreased 1.281 per cent.

As compared with  $B_2$ , the butyric acid in  $B_6$ , from cows receiving peanut oil, increased 0.455 per cent; the caproic acid decreased 0.342 per cent; the caprylic acid decreased 0.348 per cent; capric acid decreased 0.971 per cent; lauric acid decreased 1.529 per cent; myristic acid decreased 4.827 per cent; palmitic acid decreased 8.902 per cent; stearic acid increased 5.211 per cent; and oleic acid increased 11.479 per cent.

The butyric acid in  $B_8$ , from cows receiving corn oil, increased 0.603 per cent as compared with  $B_2$ ; caproic acid decreased 0.434 per cent; caprylic acid decreased 0.415 per cent; capric acid decreased 0.885 per cent; lauric acid decreased 1.659 per cent; myristic acid decreased 5.622 per cent; palmitic acid decreased 10.908 per cent; stearic acid increased 5.467 per cent; and oleic acid increased 14.194 per cent.

As compared with  $B_2$ , the butyric acid in  $B_{10}$ , from cows receiving soybean oil increased 0.224 per cent; the caproic acid decreased 0.484 per cent; the caprylic acid decreased 0.342 per cent; the capric acid decreased 0.986 per cent; the lauric acid decreased 1.371 per cent; the myristic acid decreased 5.922 per cent; the palmitic acid decreased 11.471 per cent; the stearic acid increased 5.551 per cent; and the oleic acid increased 15.078 per cent.

In brief, the coconut fat decreased all the soluble acids in increasing amounts from butyric to capric acid, increased the lauric and myristic acids, and decreased the oleic acid.

Peanut oil increased the butyric acid, decreased all the other acids from caproic to palmitic in increasing amounts, and increased stearic and oleic acids materially.

Corn oil increased the butyric acid, decreased all the other acids from caproic to palmitic acid in increasing amounts (except caprylic), and increased the stearic and oleic acids.

Soybean oil increased the butyric acid and decreased all the other acids from caproic to palmitic in increasing amounts (except caprylic), and increased the stearic and oleic acids.



The question naturally arises with butter fats  $B_8$ ,  $B_9$ , and  $B_{10}$ , from cows receiving peanut, corn, and soybean oils, as to whether the high iodine numbers might not be due in part to the direct assimilation and transference of linolic and linolenic acids from the oils fed to the butter fat. A number of determinations were made by the lead-salt-ether method with a view of obtaining some definite information on the subject, of which those in Table XXX are fairly typical.

TABLE XXX.—*Lead-salt-ether-soluble fatty acids*

Sample No.	Fatty acids recovered.	Iodine number.	Neutralization number.
	<i>Per cent.</i>		<i>Mgm.</i>
$B_8$ .....	41.86	82.11	217.94
$B_{10}$ .....	42.55	83.02	218.27

Lead-salt-ether-soluble fatty acids recovered from butter fat are rarely pure liquid acids but are more or less contaminated with small quantities of water-soluble acids extremely difficult to remove by washing and of insoluble saturated acids that pass the filter. The results indicate, however, that with due allowance the liquid acids could not have contained any appreciable quantity of higher unsaturated acids than oleic.

## II. SUMMARY OF DATA FROM MASSACHUSETTS AND ELSEWHERE, TOGETHER WITH SUCH GENERAL DEDUCTIONS AS SEEM WARRANTED

TABLE XXXI.—*Analysis of 29 samples of butter fat from Massachusetts cows fed normal rations*

	Average	Range.
Saponification number ( <i>s</i> ).....(mgm.)..	231.403	224.626 to 235.333
Acid number ( <i>a</i> ).....(mgm.)..	2.049	.927 to 3.657
Ether number ( <i>e</i> ).....(mgm.)..	229.355	222.186 to 232.530
Iodine number.....	28.461	22.720 to 36.241
Equivalent in oleic acid.....(per cent) ..	31.315	25.273 to 40.313
Total fatty acids ( <i>T</i> ) (1.00—0.00022594 <i>e</i> ) ..		
.....(per cent) ..	94.818	94.746 to 94.980
Neutralization number ( <i>n</i> ) <i>s/T</i> ..... (mgm.)..	244.051	236.498 to 248.383
Free fatty acids ( <i>A</i> ) <i>a/n</i> .....(per cent) ..	.840	.383 to 1.502
Soluble fatty acids ( <i>s</i> ) ( <i>T-I</i> ).....(per cent) ..	7.297	6.470 to 8.601
Neutralization number.....(mgm.)..	504.787	460.664 to 539.370
Insoluble fatty acids ( <i>I</i> ) by alcoholic potash ..		
.....(per cent) ..	87.519	86.145 to 88.471
Neutralization number.....(mgm.)..	222.362	215.676 to 226.310
Stearic acid by crystallization.....(per cent) ..	15.011	5.544 to 23.304
Glycerol (0.00054703 <i>e</i> ).....(per cent) ..	12.546	12.154 to 12.720
Refractive index <sup>1</sup> (Abbe) 40° C.....	1.4536	1.4534 to 1.4536
Colorimeter (Lovibond) <sup>1</sup> ½-inch cell.....	Yellow 3.2 Orange 1.4	Yellow 2.0 to 5.8 Orange 1.0 to 1.8

<sup>1</sup> Twelve samples.

The 29 samples of butter fat of grade Holsteins and grade Jerseys on normal rations, reported above, contained on the average 94.82 per cent of total fatty acids with a neutralization number of 244; 0.84 per cent of free fatty acids; 7.30 per cent of soluble fatty acids with a neutralization number of 504; 87.52 per cent of insoluble fatty acids with a neutralization number of 222.36; and 12.55 per cent of glycerol. The range in percentage and in neutralization number of both the soluble and insoluble fatty acids was rather pronounced.

TABLE XXXII.—Fatty acids in 21 samples of butter fat from Massachusetts cows fed normal rations

Fatty acids.	Average	Range.
<b>Soluble acids:</b>	<i>Per cent.</i>	<i>Per cent.</i>
Butyric acid <sup>a</sup> (by difference) . . . . .	2.932	2.241 to 4.230
Caproic acid <sup>a</sup> . . . . .	1.898	1.290 to 2.400
Caprylic acid <sup>a</sup> . . . . .	.786	.527 to 1.041
Capric acid <sup>a</sup> . . . . .	1.570	1.187 to 2.008
Total . . . . .	7.186	6.470 to 8.601
<b>Insoluble acids:</b>		
Lauric acid . . . . .	5.849	4.533 to 7.687
Myristic acid . . . . .	19.784	15.554 to 22.618
Palmitic acid (by difference) . . . . .	15.167	5.782 to 22.863
Stearic acid . . . . .	14.907	7.803 to 20.370
Oleic acid . . . . .	31.895	25.273 to 40.313
Total . . . . .	87.602	86.145 to 88.471
Total fatty acids . . . . .	94.788	94.746 to 94.980

<sup>a</sup> Twenty samples.

The 20 samples of butter fat (analyzed for fatty acids) from cows on normal rations contained on the average 2.93 per cent of butyric acid, 1.90 per cent of caproic acid, 0.79 per cent of caprylic acid, and 1.57 per cent of capric acid. The range in individual acids averaged about 85 per cent, but in most cases the differences were fairly compensating as determined by the neutralization number.

The 21 samples averaged 5.85 per cent of lauric acid, 19.78 per cent of myristic acid, 15.17 per cent of palmitic acid, 14.91 per cent of stearic acid, and 31.90 per cent of oleic acid. The range in lauric, myristic, and oleic acids was less proportionally than in the solubles, in stearic acid somewhat greater, and in palmitic acid almost 300 per cent. As the neutralization number of palmitic acid is nearly that of the average insoluble acid mixture the other four acids were fairly compensating in most instances.

TABLE XXXIII.—Fatty acids in butter fat

	Bell (1, p. 48). <sup>1</sup>	Blyth (2, p. 272).	Browne (3, p. 823).	Crowther and Hynd (4, p. 145).			Duclaux (5, p. 1024).
				Dairy butter.	First run- nings.	Last run- nings.	
Soluble acids:							
Butyric acid.....	6.13	3.49	5.45	4.45	4.30	4.06	3.38 to 3.65
Caproic acid.....		2.40	2.09	1.45	1.98	1.48	2.00 to 2.26
Caprylic acid.....	2.09	.80	.49	.99	1.11	1.37	
Capric acid.....			.32	1.10	1.51	.96	
Total.....	8.22	6.69	8.35	7.99	8.90	7.87	
Insoluble acids:							
Lauric acid.....			2.57	3.55	5.08	6.40	
Myristic acid.....			9.89	20.13	10.38	18.78	
Palmitic acid.....	49.46	47.50	38.61	15.24	17.47	11.78	
Stearic acid.....			1.83	1.08	5.93	3.19	
Oleic acid.....	36.10	40.40	32.50	45.47	46.49	41.31	
Di-hydroxy stearic acid.....			1.00	.68	.30	.16	
Total.....	85.56	87.90	86.40	86.15	85.65	81.62	
Total fatty acids.....	93.78	94.59	94.75	94.14	94.55	89.49	

	Fleischmann and Warm- bold (6, p. 302).				Jen- sen (11, p. 277).	Koefoed (12, p. 133). <sup>2</sup>	Leathes (14, p. 30). <sup>3</sup>	Molinari (17, p. 387)	Molt (13). <sup>4</sup>
	1	2	3	4					
Soluble acids:									
Butyric acid.....	5.00	5.00	4.32	4.32	3.92	1.42	5.25 to 6.12		
Caproic acid.....	2.00	2.00	2.16	2.16	1.88	1.90	90 to 2.70		
Caprylic acid.....	.15	.15	.67	.67		.47	Traces.		
Capric acid.....						1.90	Traces.		
Total.....	7.15	7.15	7.15	7.15		5.69			7.40
Insoluble acids									
Lauric acid.....						7.58	Traces.	14 to 16	
Myristic acid.....			4.46	10.00		20.86	Traces.	11 or more	
Palmitic acid.....	52.12	51.10	48.04	42.75		26.54		14 to 18	20.00
Stearic acid.....		3.35	5.54	2.00		1.90		7 to 11	40.93
Oleic acid.....	35.63	33.30	28.81	33.00		32.23		25 to 30	26.52
Arachidic acid.....							Traces.		
Higher unsaturated acids.....								4 to 5.7	
Total.....	87.75	87.75	87.75	87.75		89.11			87.45
Total fatty acids.....	94.90	94.90	94.90	94.90		94.80			94.85

<sup>1</sup> Reference is made by number (italic) to "Literature cited," pp. 397-398.<sup>2</sup> Results recalculated on a basis of 94.80 per cent total fatty acids.<sup>3</sup> Recalculated.<sup>4</sup> Results recalculated by Browne.

TABLE XXXIII—Fatty acids in butter fat—Continued

	Partheil and Ferie (18, p. 566).		Richmond (19, p. 42).	Siefeld (20, p. 202, 204, 205).				Smedley (21, p. 457).	Spallanzini (22, p. 808). <sup>2</sup>
				1	2	3	4		
<b>Soluble acids:</b>									
Butyric acid			3.43	3.53	3.58	3.27	3.35		4.44
Caproic acid			3.25	2.50	1.26	1.73	1.68		.92
Caprylic acid			.51	1.03	2.87	1.89	2.21		.28
Capric acid			1.77						
<b>Total</b>			8.96	7.06	7.71	6.89	7.24		5.64
<b>Insoluble acids:</b>									
Lauric acid	16.40	14.88	6.94						
Myristic acid	11.09	11.88	19.14	25.35	22.94	30.70	26.00		
Palmitic acid	18.24	14.46	24.48	17.18	20.84	16.89	20.96		
Stearic acid	6.65	10.49	1.72				10-15		89.48
Oleic acid	30.67	32.64	33.60	42.75	40.53	37.98	38.09		
Higher unsaturated acids	5.40	4.15							
<b>Total</b>	88.45	88.50	85.88	85.28	84.31	85.57	85.05		89.48
<b>Total fatty acids</b>			94.84	92.34	92.02	92.46	92.29		95.12
Violette (23, p. 346).									
	Superior qualities of butter.			Inferior qualities of butter.				Wright (24, p. 14). <sup>2</sup>	
	1	2	3	4	5	6	7		
<b>Soluble acids:</b>									
Butyric acid	6.07	5.33	5.50	5.03	4.62	4.80	4.76	4.37	5.25
Caproic acid	3.66	3.23	3.34	3.06	2.80	2.92	2.89	2.65	
Capric acid	2.85	3.00	2.80	3.00	2.90	2.40	3.00	2.95	
<b>Total</b>	12.58	11.56	11.64	11.11	10.32	10.12	10.65	9.97	
<b>Insoluble acids:</b>									
Lauric acid									
Myristic acid									
Palmitic acid	82.28	82.63	82.87	83.20	84.32	84.31	83.83	84.62	
Stearic acid									
Oleic acid									
<b>Total</b>	82.28	82.63	82.87	83.20	84.32	84.31	83.83	84.62	
<b>Total fatty acids</b>	94.86	94.19	94.51	94.31	94.64	94.43	94.48	94.59	

<sup>2</sup> Recalculated.

The results of other workers and compilers showed noticeable variations in total, soluble, and insoluble fatty acids (affected no doubt by the method of analysis, as well as by difference in product), but after the elimination of extremes they agreed as to the general character of butter fat better than might be expected. Although any deductions are open to criticism, the results appeared to indicate a total fatty acid content of approximately 94.41 to 94.90 per cent, soluble acids 6.69 to 8.96 per cent, and insoluble acids 86.15 to 88.50 per cent. These figures do not differ materially from those secured on Massachusetts samples.

The percentage of the different soluble fatty acids by other investigators showed decided variations. The results, while not strictly comparable, recognized butyric acid as the most prominent, with caproic acid second. The data for caprylic and capric acids were quite limited. In a few instances caprylic acid was shown to be the smallest constituent,

but in no case did both caprylic and capric acids agree with the average of the samples tested in Massachusetts.

Of the insolubles, lauric acid was seemingly at least one of the smaller and myristic acid one of the larger ingredients, but only in a few cases were the results at all consistent. The range in palmitic acid was rather wide, but on excluding the higher percentages most of the remainder were between 15 and 25 per cent.

In stearic-acid content there was again considerable divergence. The results may be divided into two groups from 1.08 to 3.35 per cent and from 5.54 to 15 per cent. Of the more recent workers Miss Smedley obtained a higher percentage than the others. The Massachusetts average herein reported on normal rations is somewhat higher than previously secured (9, *p.* 109-110) and may prove higher than subsequent figures from a greater number of cows will justify.

The percentage of oleic acid varied from 25 to 46.49. A number of the foreign butter fats had an oleic-acid content similar to those derived in Massachusetts from feeding corn and soybean oils. This acid appears to respond the most readily to the influences of feed and advancement in lactation.

The small quantity of di-hydroxy stearic acid reported in butter fat may arise from decomposition changes accompanying analytical procedure rather than as a natural occurrence. The weight of evidence does not support the contention that unsaturated acids higher than oleic are present in straight butter fat to any appreciable amount.

#### GENERAL CONCLUSIONS

(1) On normal rations the percentage of total fatty acids in the butter fat of the Holsteins was substantially the same as that of the Jerseys, but their neutralization number was somewhat lower; the free fatty acids and soluble fatty acids were also lower, while the percentage of insoluble fatty acids was higher and their neutralization number and glycerol lower.

(2) The percentages of butyric and caprylic acids were lower in the Holsteins than in the Jerseys; caproic and capric acids higher; lauric and oleic acids higher; and myristic, palmitic, and stearic acids lower.

(3) With advancing lactation the percentages of total fatty acids increased slightly in both breeds, while their neutralization numbers decreased; the soluble fatty acids decreased, while the insoluble fatty acids increased and their neutralization numbers and the glycerol decreased. As a rule the changes were more pronounced in the Holsteins.

(4) The addition of oils and fats to the normal rations increased the insoluble fatty acids, partly at the expense of the solubles and partly by increasing the total fatty acids, and in consequence depressed the glycerol content.

(5) The neutralization numbers of the soluble, insoluble, and total fatty acids were decreased on feeding the peanut, corn, and soybean oils, and that of the insoluble acids was increased by coconut fat.

(6) Of the soluble acids, butyric fluctuated but maintained its percentage, caproic acid decreased appreciably, and caprylic and capric acids decreased about 50 per cent in extreme cases. Of the insoluble acids, lauric and myristic acids increased when coconut fat was fed but decreased when unsaturated oils were fed. In the latter cases oleic acid increased greatly.

(7) In so far as it is possible to draw a general conclusion from the data thus far secured from all experiments (15, 16) made at this station extending over many years, it seems evident that neither protein nor carbohydrates have any appreciable influence in changing the chemical composition of the butter fat; on the other hand, different oils and fats do modify to an extent the chemical composition of butter fat, the modification depending upon the composition of the oils and fats fed.

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# STRIPED SOD WORM, CRAMBUS MUTABILIS CLEMENS<sup>1</sup>

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## INTRODUCTION

Throughout a wide area *Crambus mutabilis* is one of the most common species of the genus. It ranks well toward the head of the list in destructiveness, although by itself it never has been directly charged with a destructive outbreak. It has not previously received detailed study, and the available information concerning it is scattered and meager. The present paper includes a summary of previously published facts, together with the results of the writer's studies for several years.

## SYSTEMATIC HISTORY

*Crambus mutabilis* was first described by Clemens (3, p. 204)<sup>2</sup> in 1860, but he furnished no information as to the source of his material. Three years later Zeller (15, p. 44) redescribed it as *Crambus fuscicostellus*, a name which better characterizes the species than Clemens's adjective. Both names appear in the literature for some years, although Grote (7, p. 79) early recognized their probable synonymy. Smith (13, p. 87) first placed *fuscicostellus* unconditionally as a synonym of *mutabilis*, in which he is fully borne out by Hampson (8, p. 928), who had Zeller's type in the British Museum for comparison.

## GEOGRAPHICAL DISTRIBUTION

*Crambus mutabilis* seems to be a purely North American species, for outside of North America it has been reported only by Hedemann (9, p. 300), from St. Thomas Island in the West Indies. It is widespread over the eastern half of the United States. A study of the published records and of all the available museum material shows that the outlying points from which reliable records are available are Brownsville and Amarillo, Tex.; Vineyard, Utah; Sioux City, Iowa; and Cartwright, Manitoba. It has been found in most of the States to the eastward of a line connecting these places, although it does not appear in a considerable collection of the genus made in southern Minnesota, and has not been recorded from Wisconsin, West Virginia, and several of the New England States.

The reported occurrence in Nebraska is evidently based on Bruner's paper (2, p. 262), in which, however, he does not say that it has been taken in that State. It has been taken at numerous points in Florida, but, except for a single specimen from northwestern Arkansas, has not yet been reported from the tier of States between and including Oklahoma

<sup>1</sup> Accepted for publication July 11, 1922. This paper is the third in a series of Contributions to a Knowledge of the Crambinae of North America. I. *Crambus hemiochrellus* Zeller, appeared in Annals of the Entomological Society of America for March, 1918, and II. *Crambus laqueatellus* Clemens, in the June, 1922, issue of the same journal.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 414.



and South Carolina, although it probably occurs throughout that section. There is a specimen in Doctor Barnes's collection from Digby, Nova Scotia. It has also been reported from California in a broad way, by various writers following Felt (4, p. 97), but the writer has seen nothing to substantiate this record. There is a single specimen in the National Museum labeled "Arizona," from Riley's collection. The accompanying map (fig. 1) shows the points in the United States whence definite records have been obtained. Where no exact locality is given the State is marked with an interrogation point.

#### FOOD PLANTS

The striped sod webworm has never been recorded as feeding on any plant outside the grass family. In the field, larvæ have been taken on blue grass, corn, wheat, timothy, and once, at Nashville, Tenn., on small clumps of a wild grass which was not at the time in determinable con-

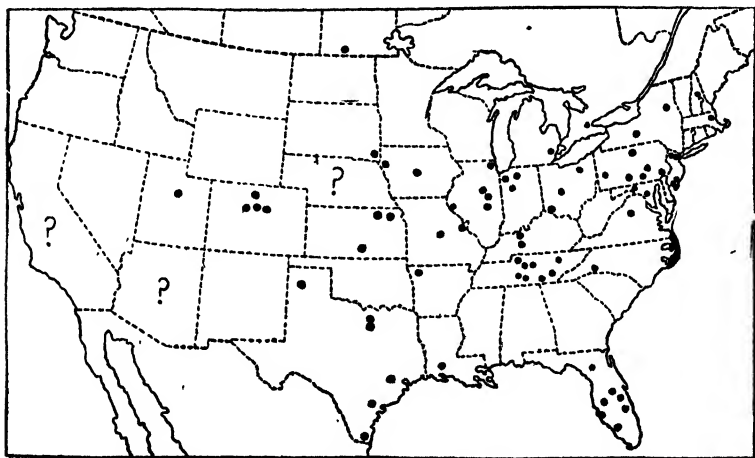


FIG. 1.—Map showing known distribution of *Crambus mutabilis* in North America.

dition. In addition to the foregoing the writer has reared it in cages on crab grass (*Syntherisma sanguinalis*), barley, and rye. Larvæ were also offered oats and orchard grass (*Dactylis glomeratus*) and in the later stages fed freely. The young larvæ, however, seemed to have trouble in subsisting on these grasses, and none were reared on them alone. Undoubtedly the larvæ will thrive on many other grasses.

#### ECONOMIC HISTORY

Few cases of serious injury are definitely chargeable to *Crambus mutabilis*. This does not mean that it is not an injurious form, but merely that it has not been caught in the act. There is plenty of circumstantial evidence to warrant an indictment aside from the established records. Bruner (2, p. 262) first mentions it as a grass insect attacking corn following sod. Riley (11, p. 36) says it occasionally attacks corn. Webster (14, p. 86) intimates that this species may have had something to do with the destruction wrought in meadows and in

corn and oat fields in northern Ohio in 1895 by his statement that the moths were taken in June following the outbreak, but "in more limited numbers" than those of *C. trisectus*, to which the larger part of the trouble was undoubtedly due. From Forbes (6, p. 39-42) there are numerous reports of crambid injuries, especially to corn, but in not a single instance does he attribute the damage to this species alone; always at least one other species is involved.

In the writer's experience larvæ have been found in numerous instances destroying corn plants, and almost invariably either the crop followed sod or the injury was confined to the margin of the field. At Spring Hill, Tenn., one larva was found destroying a wheat plant early in June. A. F. Satterthwait has on three occasions sent the writer larvæ found feeding in the bulbs of timothy plants. In some experimental plantings of corn on sod land near Chapel Hill, Tenn., three of these larvæ were found to every one of *Crambus caliginosellus*, the only other crambid present, and the stand of corn was being very materially injured by them. In 1920, near La Fayette, Ind., this species, associated with *C. trisectus*, caused severe injury in several fields of corn.

Although there is no record of a general outbreak of this species, it is certain that, with favoring conditions, severe but narrowly local outbreaks occur more often than is realized. The moths are often sufficiently numerous to show that a large amount of food has been required to produce them, and as the larvæ seem to be strictly grass feeders the species must be placed well toward the head of the list of injurious forms. It seems especially subject to parasitism and epidemic diseases, and this may explain why it has not increased to such an injurious degree as some of the other species.

#### SEASONAL HISTORY

In Tennessee, where collections have been constantly made for the last five years, the moths first appear about May 15, the date of their appearance being very uniform year after year. By May 20 they are abundant and continue so for a month in gradually decreasing numbers. During five years' collecting no moths have been taken between June 19 and July 3, and only scattering individuals until about July 10, when the moths of the second generation appear in greater numbers and remain over a longer period than those of the first generation, continuing fairly abundant until August 15. Then after another interval of absence a few appear during the last few days of August and in September, evidently a partial third generation. The latest record for the country is of one moth taken at Knoxville, Tenn., October 26, long after the others were gone.

In Florida, the moths of the first generation appear as early as the latter half of February, but data are lacking for the rest of the year. A fairly complete series of collections made at La Fayette, Ind., shows that in that latitude the periods of abundance fall about a month later than in Tennessee, the first from June 20 to July 10 and the second from August 6 to September 8. Even there the partial third generation appears, as moths were taken October 1 and 2 after having been absent for nearly a month. The La Fayette records accord closely with Forbes's (6, p. 43) statement for Illinois—

two well-marked periods of maximum occurrence, one in July and one in August, with a comparatively sparse showing toward the middle of July.

Both Felt (4, p. 65) and Slingerland (12, p. 210) report the results of trap lantern collections at Ithaca, N. Y., and, although the number of moths taken is too small to more than indicate the facts, they seem to show that the two periods of greatest abundance lie between June 10 and 20 and July 8 and 20. More than one month is required for the development of a generation, so these Ithaca figures must be taken subject to revision.

### REARING RECORDS

Laboratory records fully bear out the foregoing observations. Table I is a compilation of all the rearing records available for individuals under observation from egg to adult. It will be noticed that during the summer the interval between oviposition and the emergence of the adult is from 42 to 70 days, averaging about 55 days. Allowing three days for the preoviposition period brings it to 58 days, slightly less than two months from egg to egg. This works out almost exactly in the table; one generation from May 23 to July 21; the second from July 21 to September 11; and the third, or overwintering generation, from September 11 to the following May. It will be noted also that many of the later larvæ of the second generation, those coming from eggs laid after August 1, emerged the following spring. Thus it is safe to conclude that in Tennessee there are normally three generations per year, some of the second and all of the third overwintering as larvæ. Farther north the third doubtless becomes smaller and may completely disappear, leaving larvæ of only the second generation to pass the winter.

TABLE I.—Rearing records of *Crambus mutabilis* under observation in the laboratory or insectary from egg to adult<sup>1</sup>

Cage No.	Eggs laid.	Moth out.	Number of days.	Sex.	Number of moths.
A . . . . .	May 23	July 8	46	♂	1
A . . . . .	23	Aug. 5	74	♂	1
1775 . . . . .	23	July 30	68	♂	1
1775 . . . . .	23	Aug. 2	71	♀	1
B . . . . .	26	July 7	42	♂	1
C . . . . .	26	8	43	.	1
C . . . . .	26	Aug. 5	71	♂	1
E . . . . .	27	July 11	45	...	1
1777 . . . . .	30	31	62	♀	1
15268 . . . . .	June 3	26	53	♂	1
15284 . . . . .	6	26	50	♂	1
H . . . . .	9	31	52	♂	1
H . . . . .	9	Aug. 12	64	♂	1
G . . . . .	12	July 31	49	♂	1
1792 . . . . .	12	Aug. 2	51	♀	1
I . . . . .	16	12	57	♀	1
J . . . . .	July 21	Sept. 3	44	.....	2
J . . . . .	21	4	45	.....	8
J . . . . .	21	5	46	.....	5
J . . . . .	21	7	48	{ 6 ♂ 6 ♀ }	12
J . . . . .	21	8	49	{ 1 ♂ 1 ♀ }	2
J . . . . .	21	9	50	{ 1 ♂ 2 ♀ }	3
J . . . . .	21	14	55	♂	1

<sup>1</sup> The records designated by letters were made in 1924; in other cases the first two figures of the cage number indicate the year in which the records were made.

TABLE I.—Rearing records of *Crambus mutabilis* under observation in the laboratory or insectary from egg to adult—Continued

Cage No.	Eggs laid.	Moth out	Number of days	Sex.	Number of moths.
J .....	July 21	Sept. 23	64	♂	1
15406 .....	24	11	49	♂	1
15406 .....	24	13	51	5 ♂ 4 ♀	9
15406 .....	24	14	52	1 ♂ 3 ♀	4
15406 .....	24	17	55	1 ♂ 1 ♀	2
15414 .....	27	18	53		2
16221 .....	29	22	55	♂	1
16221 .....	29	24	57	♂	1
16221 .....	29	29	62	♂	1
16221 .....	29	Oct 1	64	♀	1
16221 .....	29	3	66	♂	1
16221 .....	29	17	80	♂	1
16221 .....	29	19	82	♀	1
16221 .....	29	Nov. 6	98	♀	1
16221 .....	29	14	102	♀	1
16221 .....	29	Apr 23	268	♀	1
16221 .....	29	May 21	296		1
15476 .....	Aug. 6	Oct 19	74	♀	1
16260 .....	11	Apr 23	255		2
16259 .....	16	May 2	259		1
16259 .....	16	21	278		1
16259 .....	16	27	284		2
R <sup>2</sup> .....	Sept 11	Dec 9	58	♀	1
15607 .....	12	May 26	256	♂	1
15602 .....	17	June 2	258	♀	3
15619 .....	27	1	247	♂	2
15619 .....	27	5	251	♀	1

\* Reared in laboratory

Table II is a record of all the rearings of larvæ collected in the field, showing in most cases the interval between the activity of the larvæ in the spring and the appearance of the moths of the first generation. The data apply to middle Tennessee unless otherwise stated.

TABLE II.—Rearing records of *Crambus mutabilis* larva collected in the field

Cage No.	Larva taken.	Moth out	Sex.	Source.
182 .....	Apr. 3	May 25	♂	Grass roots, fed blue grass.
15141 .....	20	24	♂	Blue grass.
1655 .....	21	June 27	♀	Grass.
1824 .....	29	3	♀	Corn.
12358 .....	May 9	13	♀	Corn.
Field note .....	10	.....	.....	Clump grass; not reared.
16104 .....	10	May 29	♂	Grass
12358 .....	15	June 8	.....	Corn.
15359 .....	July 7	July 27	♀	Blue grass.
15359 .....	7	Aug. 9	♀	Blue grass.
C999G .....	27	Sept. 10	.....	Timothy, Athens, Ind
C1037 .....	Aug. 6	5	♂	Timothy, Nortonville, Ky.
15641 .....	Sept. 30	.....	.....	Timothy, La Fayette, Ind.; not reared.
15687 .....	Nov. 9	.....	.....	Blue grass, Nashville, Tenn.; not reared.

A series of 50 larvæ from eggs laid July 23 was reared for individual instar records. Each larva was confined in a half-ounce tin box floored with damp blotting paper and was fed blue grass. A record was made of each molt, and the results are condensed in Table III.

TABLE III.—*Instar records of 50 Crambus mutabilis larvæ from eggs laid July 23, 1915*

Stage	Maximum.	Minimum.	Average.	Number of individuals included in average.
	Days.	Days.	Days.	
Egg.....	7	7	7	.....
Larva:				
Instar I.....	5	3	3.2	50
II.....	5	2	3.3	48
III.....	4	3	3.3	44
IV.....	5	3	3.3	41
V.....	7	3	4.1	36
VI.....	7	3	4.8	29
VII.....	17	9	14.1	19
Pupa:				
♂.....	11	7	9.9	8
♀.....	10	7	8.0	8
Total.....	56	50	52.6	16

## THE MOTH

### HABITS

The moths of this species (fig. 2) are essentially grass lovers and are seldom found among weeds or bushes. They are reluctant to leave tall grass for a mown field or closely grazed pasture and when driven out will at once circle back.

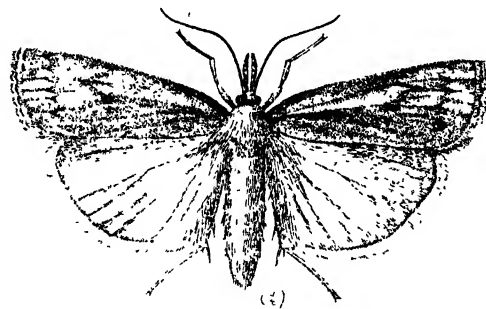


FIG 2.—*Crambus mutabilis*: Adult female. About three times natural size.

They alight abruptly, usually on a grass stem, and instantly turn head downward and stand with the head pressed closely to the stem and the body elevated at a considerable angle. When at rest the dark costal margin of the forewing contrasts with the paler gray median portion and with the pale gray underbody, giving the moth an almost

striped appearance when viewed from the side. This coloring and the characteristic attitude make this species one of the easiest to identify at a distance in the field. The moths are most often found in abundance in the lower and damper portions of the meadow, seemingly attracted by the greater luxuriance of the grass. They also occur in open woodland, especially if the ground is grass-covered. Their flight is erratic and awkward.

All-night collections at light made throughout the summer in 1915 show that the female moths are attracted during the early part of the evening, between 8 and 9 o'clock and the males not until later, between midnight and 3 a. m. Very few of either sex appeared outside their respective periods.

It was proved by numerous experiments that the moths will feed on dilute honey and will drink water, but that food is not necessary and that they will live longer and the females will produce more eggs on the average when supplied with water alone than when fed honey or when confined in a dry box or vial. Moths in the field have never been observed feeding, or attracted to flowers.

The average length of life of 72 female moths taken at light and in the field and confined, some in dry boxes and some with wet cotton, was 3.86 days. Under the same conditions 115 males averaged 1 day less. One reared female, which had access to water from the time she emerged, lived 10 days and laid 500 eggs, which is probably about normal for a moth in the field. Under optimum conditions 3 males lived 14 days each, but a summary of all records shows that the males are usually shorter-lived than the females.

Practically all eggs obtained from moths taken in the open are fertile, indicating that mating occurs very shortly after the emergence of the females, probably the same night. Occasionally the last few eggs laid by a female prove to be infertile, but in most cases one mating is enough to fertilize the entire supply of eggs, and there is no evidence that the moths normally mate more than once. Female moths isolated and prevented from mating usually deposit some eggs, but these invariably shrivel and fail to hatch.

#### DESCRIPTION (FIG. 2)

Wing expanse 18 to 24 millimeters, the females averaging larger than the males. General color gray, with a dusky spot near center of forewing, the inner half of costal margin dark brown. Palpi fuscous, the tips of the scales whitish. Head and thorax gray-brown. Male antennae broadly pectinate (Pl. 2, A), female setaceous. Forewing with costal half slaty gray, sometimes whitish toward the center, anal half with a tinge of luteous. Proximal half of costal margin broadly bronze-brown. A dark brown median line begins near the middle of costal margin, forms a broad angle near end of cell, broadens immediately below it, and continues in an oblique line, gradually narrowing until it reaches the hind margin. In feebly marked specimens this median line is often obsolete except the portion below the end of the cell, which is invariably present as a more or less conspicuous dusky spot. Subterminal line runs nearly straight across the wing, with an acute outward angle at each vein. Terminal line of seven dusky spots at the ends of the veins. Fringes gray, shining. Hind wings gray, a little paler toward base, fringes pale yellow.

**GENITALIA.**—Male: Body of tegumen (Pl. 2, C) rather long, a little longer than the uncus, rounded above, its limbs long, narrow, turned ventrad, and narrowed at the ends. Uncus nearly straight, rather narrow, with a sharp, nail-like hook at the end, hirsute above. Gnathos long, slender, much exceeding the uncus, tip narrowed and turned slightly ventrad, naked. Aedoeagus (Pl. 2, E) straight, cylindrical, smoothly rounded cephalad, tapering somewhat from the opening to the tip, which flares slightly, terminal opening oblique, with a single long slender heavily chititized cornutus, about half the length of the organ; anellus reduced to a mere membranous scale on ventral side. Harpes (Pl. 2, D) small, rather weakly chititized, free costa reduced to a slender sharp spine less than half the length of the free sacculus, outer margin at base hirsute; sacculus with the free portion a flat curved process with rounded tip, hirsute, the hairs on ventral half much shorter than those above, narrowed at base, and with a rounded spined lobe where it joins the base. Vinculum reduced to a small scutate plate lying between the tips of the base of the sacculi. Female: The ventral two-thirds of the genital plate (Pl. 2, F) rounded and somewhat produced, the dorsal lobe smaller, rounded, both lobes hirsute.

## THE EGG

## DESCRIPTION

Elliptical in outline, bluntly rounded at the ends, one of which is very slightly more obtuse than the other. Length, 0.494 to 0.441 millimeter, average, 0.479 millimeter, width, 0.318 to 0.265 millimeter (average, 0.306 millimeter). The chorion is ornamented with prominent acute longitudinal ridges, usually 17 in number but varying from 16 to 20. These ribs become obsolete at the ends of the egg, where the polar disks are only slightly tuberculate; in the intervals between the ribs are much smaller cross striae, about 30 in the length of the egg (Pl. 1, C).

The eggs laid by one female are very constant in size and shape, but there is enough variation between those from different moths to make the measurements of little value for specific determination.

The eggs are very pale cream, almost white when laid, but they soon deepen in color, reaching a pale salmon yellow in about three days, after which, until maturity, the color remains unchanged. After the third day the minute dark eye spot is visible within the egg. A few hours before hatching the darkening of the head and thoracic plate gives a purplish tinge to the whole mass of eggs. The egg is cut slightly to one side of the larger end and the larva escapes through a more or less ragged opening, leaving the parchmentlike shell nearly transparent.

## NOTES

In summer the eggs hatch in from five to seven days, the variation evidently depending on the temperature. When fully developed the larva leaves the egg regardless of outside conditions and does not remain quiescent as do some others of this group. The eggs are perfectly dry and nonadhesive when laid, and fall down among the grass stems as they are dropped by the moths either in flight or at rest. Their small size and lack of definite location make them hard to discover; and to find one a prolonged search is necessary even when they are known to be abundant.

Dissection of two freshly emerged moths showed, respectively, 560 and 1,120 eggs and egg cells in the ovaries. In the latter case the abdomen also contained a large amount of fat in the form of small bodies about the size of the matured eggs, but whiter and more irregular in shape. The first moth showed a much smaller quantity of this reserve supply, which is probably accounted for by less favorable feeding conditions during the latter part of the larval life. This partial starvation of the larva seems to affect directly and very decidedly the fecundity of the moths.

So far as the writer has been able to determine from many experiments with this and other species of *Crambus*, the females do not require food other than water for the development of the immature ova in the ovaries at emergence. Spent moths show an entire absence of fat bodies and immature ova, the youngest of these having evidently broken down and been used for the nutrition of the larger eggs. So while there were 1,120 eggs and egg cells in the more fecund of the two moths mentioned above, the actual number of eggs which she would have matured would have been much less than that, perhaps 700, and not over 350 for the other. The largest number of eggs produced by a single moth of this species, of which the writer has record, is 753 eggs, and very few attained 500. The average number produced by moths taken in the field and at light was 170, but many of these had doubtless laid part or all of their supply before capture.

## THE LARVA

## HABITS

The larva of *mutabilis* is one of the easiest to recognize in the field. The distinct striping of the body and the prominent dark markings of the head distinguish it. When newly hatched the head and cervical plate are shining jet black<sup>3</sup> and the body pale grayish yellow except for the spot of color in the intestinal tract, due to the bit of eggshell eaten in leaving the egg. The pinacula are dusky and unusually conspicuous for the first instar. During the first three instars the head remains black or very deep fuscous. In the fourth and succeeding instars it becomes brownish yellow, marked in a definite pattern with close groups of small, round, dark brown or black spots.

In the writer's experiments many larvæ were reared on blue grass, small sods of which were potted and covered with lantern globes. The newly hatched larvæ begin to feed by cutting small pits between the vascular bundles on the upper surface of the leaf blade, usually toward the base. These pits are soon covered with a few silk fibers, and shortly the larva is concealed by excrement placed systematically in this webbing. During the first three instars, only the green leaf tissue is consumed, leaving the lower epidermis intact; thereafter the entire thickness of the leaf is eaten. As soon as the larva becomes too large to remain sheltered on the leaf blade it descends to the ground and makes a burrow lined with silk and opening usually close beside the stem. In the field there is often a valve-like arrangement at the tip of this tube, so that the larva from within can close it and remain secure from intrusion. Beneath the surface the burrow may run at almost any angle. If the earth is soft it frequently is parallel with and close beside the main stem of the plant; if the ground is hard, it may run off at right angles just beneath the surface. The upper part of the tube is rather substantially constructed and closely lined with silk. Farther down the silk is more sparingly used and often just above the lower end ceases altogether, leaving only the earthen walls. The extreme lower end is again lightly lined with silk. This peculiar construction serves a very useful purpose in protecting the larva. When the plant is disturbed the larva instantly retreats to this silk-lined extremity of its burrow. If the plant and burrow be dug or pulled from the ground the tube breaks at its weakest point. The larva draws the earth-covered silk lining tightly about itself and so closely resembles a mere lump of earth that surprisingly often it escapes further detection. These larvæ become very large and brightly colored and but for the protection of this bit of webbing would be the most easily discoverable of the webworms.

Although in the cornfield the burrow often runs into the ground close by the stem of the plant, very little if any feeding is done beneath the surface. Quite unlike *caliginosellus*, which occupies a half-cylindrical tube lying against the stem and feeds exclusively underground, *mutabilis* always has an exit to the outside and feeds aboveground. The leaves are eaten, beginning at the base, and as they are cut fall away from the plant and lie wilting on the ground. Sometimes so many leaves are cut in this way that to obtain green food the larva is forced to consume

<sup>3</sup> Felt's (4, p. 65) surmise as to variations in the color of the head of the newly hatched larva is clearly an error, for the head colors of the first instar of the various species are remarkably constant



the main stem of the plant, working from the top downward. In such cases the injury closely resembles cutworm work. Only the first-generation larvæ work on corn, and then usually only when it is very small.

In sod the injury is not so characteristic. The larva merely lives in its silk-lined tube, cuts off one blade of grass at a time, and draws it down into the burrow, where it is consumed at leisure. It is doubtful if the larvæ leave their burrows in the daytime, but they will feed during the day, as can be seen by the motion and gradual disappearance of a grass blade inserted into the burrow. All excrement is packed into the lower end of the burrow or sometimes into side pockets forking from the main burrow. When one burrow becomes too small, or is filled with the bright green sawdustlike frass, it is abandoned and another constructed. Occasionally a burrow is found as a silken tube running back under a board or stone lying on the surface of the ground.

Felt (5, p. 69-74) and other writers have described and figured definite cylindrical nests made by crambid larvæ, but these have been observed by the present writer in only one instance, and that in connection with this species. In this case numbers of larvæ were confined in lantern-globe cages with small potted sods of blue grass. They had reached about the fifth instar in late September, and at once constructed tubular nests of bits of dry grass blades tightly fastened together and smoothly lined with silk. They were suspended among the leaves of the blue-grass plants, some of them partly in the ground and some entirely above and clear of the ground. (Pl. 1, A.) They contained no excrement and were closed at the bottom, but open at the top. The larvæ seemed to make no effort to close them and wintered successfully in them, although exposed to every change of temperature in an open outdoor insectary. After the construction of these nests the larvæ fed no more until the following spring. It seems probable that such nests are constructed only when the soil is excessively wet, in order that the larva may remain dry during the cold weather. Excessive moisture and dryness are both enemies of larvæ overwintering in cages, and it is difficult to provide exactly proper conditions for them. From these and other observations it may be concluded that in Tennessee the larvæ construct and enter their winter quarters about the last of September. They begin feeding again in April, and the first moths make their appearance about the middle of May.

Two series of 10 larvæ each were run at different times to obtain records of the amount of food eaten. Blue-grass leaves cut into 30-millimeter lengths were used for food. At the close of each instar the uneaten portion was removed and measured. Since the larvæ skeletonize the leaves during the first three instars of their life and the total amount consumed during that time is insignificant, these instars are omitted from the record. The figures represent linear millimeters of blue-grass leaves of an average width of about 3 millimeters. The results of the two series were so similar that they are combined in Table IV.

As indicated by this somewhat incomplete record, the voracity of the larvæ of this species is low compared with that of *Crambus trisectus*, in which the average total consumption amounted to over 2,000 millimeters. This may be another reason why this species has not caused as serious injury as some of the others.

TABLE IV.—Record of food eaten by larvæ of *Crambus mutabilis*

[Linear millimeters of blue-grass leaves, average width about 3 millimeters.]

Instar.	Maximum	Minimum.	Average.	Number of larvæ.
	Mm.	Mm.	Mm.	
IV.....	60	5	33	20
V.....	135	50	93	20
VI.....	480	35	183	19
VII.....	1, 140	90	496	18
VIII.....	576	68	268	4
Total.....			1, 073	....

The normal number of instars for *mutabilis* appears to be seven. Two larvæ pupated from Instar VI, but neither lived until emergence. The males and females develop in very nearly the same time, showing a difference of only 0.2 of a day between the averages for 8 of each sex.

The width of head and average length of the larva in each instar are shown in Table V.

TABLE V.—Larval measurements of *Crambus mutabilis*

Instar.	Number measured.	Head width.			Body length.
		Maximum	Minimum	Average.	
		Mm.	Mm.	Mm.	Mm.
I.....	8	0. 194	0. 194	0 194	1 7
II.....	6	. 301	. 301	. 301	2. 8
III.....	4	. 459	. 424	. 441	3 0
IV.....	5	. 582	. 547	. 570	5. 0
V.....	5	. 900	. 812	. 847	9. 0
VI.....	11	1. 306	1. 166	1. 227	13. 0
VII.....	3	1. 912	1. 586	1. 730	18. 0

## DESCRIPTION

INSTAR I.—Head shining black, cervical plate fuscous, prothorax a little darker than abdomen, mesothorax and metathorax concolorous with it. Abdomen pale transparent yellow. Pinacula on thorax and abdomen dusky and conspicuous, unusually so for the first instar in this genus.

INSTAR II.—Head shining black, frons deep fuscous, cervical plate castaneous, a little paler and more reddish than head. Rest of thorax and abdomen pale yellow. Pinacula dusky and conspicuous, for each is surrounded by a small area of brownish overcolor.

INSTAR III.—Head shining black, frons paler, fuscous, cervical plate fuscous, rest of thorax and body pale, tinted by the ingested food, skin finely granular. Pinacula brownish, rugose, more or less conspicuously surrounded by a brownish area. Caudal plate pale with dusky dots.

INSTAR IV.—[No description obtained.]

INSTAR V.—Head dark yellow with clearly defined yellowish brown markings made up of round spots arranged in broken groups but in a definite pattern as follows: One area bordering the vertical suture and extending down on to the face in a branch each side of the frons; another larger triangular area with its base on caudal margin of head, extending forward until its tip joins the forks of the vertical spot, below this a smaller crescent-shaped area midway between the frons and caudal margin of

head, and below this a line running from the ocellar area to the caudal margin of the head. This pattern remains constant throughout the remaining instars. Cervical plate dusky yellow with small dark spots. Body color pale greenish yellow tinged with a faint claret or maroon overcolor. There are four longitudinal rows of whitish spots, one on each side of the narrow middorsal line and a broader one on each latero-dorsal aspect above the spiracles, giving the larva a striped or spotted appearance. Venter pale green, skin finely granular and glistening. Pinacula dark yellowish brown, rugose.

INSTAR VI.—Head dusky yellow with conspicuous groups of round fuscous spots; frons concolorous with head. Cervical plate yellowish brown, with several dark spots. Rest of thorax and abdomen entirely covered with the brown overcolor except for the four longitudinal pale or greenish lines which are more conspicuous in this instar. Pinacula darker than body, nearly concolorous with dark markings on head. Black cicatrices on the pedal segments of the abdomen about the size and shape of the spiracles. Caudal plate dusky yellow with dark markings.

INSTAR VII.—Head as in Instar VI with the markings more sharply defined. Cervical plate dusky yellow with a faint, dark-bordered pale median line, otherwise as in Instar VIII.

INSTAR VIII.—Head dusky or amber yellow, with dark markings composed of close groups of round yellowish brown spots arranged as described under Instar V. Frons dusky yellow, outlined with a very fine dark line and outside a pale V, which at apex continues caudad to the vertical suture. Cervical plate dusky yellow, with narrow, dark-bordered pale median line and some small dark markings. The four rows of irregular whitish spots give the larva a distinctly striped appearance. Pinacula yellowish brown, leathery, shining, rugose. Cicatrices larger than spiracles, black. (Pl 2, B)

## THE PUPA

### DESCRIPTION

The pupa is pale yellow when first formed, soon changed to a golden yellow, the head, thorax, and wing cases darkening as it approaches maturity. Spiracles are present on abdominal segments 3 to 9, inclusive, those on segment 3 almost under the edge of the wing cases, those on segments 5 to 8 elevated, the one on segment 9 pale and merely a scar. Anal process rather narrow (Pl 2, G), a dorsal rounded ridge of nearly uniform width running to the rather truncate and downwardly bent tip. At the angles of this tip stand the setae of the dorsal pair, very small, depressed, and inclined cephalad. At each side of the dorsal ridge is a flattened depressed area cut by the deep, wide, curved nasal groove at whose caudal end stands a small tubercle. Ventrals this anal process is more tapering and ends in a rounded elevation from the side of which arises the ventral pair of setae, somewhat larger than the dorsal pair and diverging. Cephalad of this terminal elevation are four or five shallow, parallel, longitudinal depressions as if made by fingers laid side by side. The tip of the abdomen beneath is flattened but not concave.

### THE COCOON

When fully grown the larva makes its cocoon (Pl. 1, B) either by walling off a section of its burrow or constructing a separate chamber near by in the soil, more often the latter. The cocoon is about 15 millimeters long and half as wide, shaped like a peanut meat, rather firm to the touch, lined with soft gray silk and outwardly covered with earth particles so that it is not easily found. It lies close to the surface of the ground and when buried deeper has an extension reaching the surface. In emerging the moth leaves the pupal shell entirely within the cocoon.

### SYSTEMATIC RELATIONSHIPS

*Crambus mutabilis* appears to be the ultimate of one series in this genus. It differs from *hemiochrellus* (1, p. 57), its nearest relative, in the greater development of the antennae and the reduction in the male genitalia. The male antennae are more strongly pectinate than those of *hemiochrellus*; in fact, more so than in any other species of the genus which the

writer has seen. Each of the median segments bears from 14 to 18 sensoria (Pl. 2, A) compared with 8 or 10 in *hemiochrellus*. The male genitalia give the best basis for comparison. The free costa, which in *hemiochrellus* is a stout naked spine as long or longer than the sacculus, is in *mutabilis* a small slender spine less than half the length of the sacculus, which remains practically the same. The tegumen and uncus retain much the same shape, but the cornutus (Pl. 2, E) in the aedoeagus is both shorter and more slender than in *hemiochrellus*. The wing shape and the general uniform coloration remain similar in the two species, and the eggs of the two assume and retain exactly the same color during the incubation period.

#### NATURAL CONTROL

That this species has not more frequently been recorded as a destructive pest is probably due to two factors—the apparent great susceptibility of the larvæ to disease, and the attacks of parasites.

#### DISEASES

While making intensive studies of this species at Nashville, Tenn., in rich blue-grass meadows, where the larvæ were known to be abundant, areas were often found in which practically every burrow contained only the flaccid dead body of the maker. The same disease, evidently bacterial, was met with in the laboratory, and it was only by using the strictest care in the sterilization of the tin boxes used as rearing cages that it was possible to bring the larvæ to maturity. The disease first manifests itself in the lack of appetite and sluggishness of the larva. The next day the larva is dead and somewhat softened, but not externally changed. Another day reduces it to a shapeless sack filled with a dark brown semiliquid. Finally the skin also breaks down and the mass gradually dries up, leaving only a dark stain. The writer has not succeeded in getting a determination of the cause of the disease, but that it plays a very large part in keeping this species from becoming a serious pest can not be doubted.

Another disease which is less common kills the larva more gradually. It usually begins at the caudal end and leaves it corky in texture and densely filled with a mass of whitish hyphæ. The fungus causing it has been determined by Dr. A. T. Speare as a species of *Isaria*.

#### PARASITES

Because of the fact that the larvæ of this species have a more open method of feeding than some of the others, they appear to be more subject to the attacks of parasites than those which remain more constantly underground.

Two species of tachinid flies have been reared from larvæ of *Crambus mutabilis*, namely *Phorocera claripennis* Macq. and *Exorista nigripalpis* Towns., neither of which apparently has heretofore been recorded from this host. Both were reared from larvæ collected by W. H. Larrimer in connection with an outbreak of webworms near La Fayette, Ind., in June, 1920, in which two species of *Crambus* were concerned, *C. trisectus* and *C. mutabilis*, the former predominating to the extent of about 80 per cent of the total.

Fifteen flies of the species *Phorocera claripennis* Macq. were reared from 14 host larvæ, two of them maturing, in one instance, from one host.

One came from a larva determined before its death as *Crambus mutabilis* and the others all from mixed larvæ specifically undetermined. It is therefore unknown whether this parasite is limited to the single host or divides its attentions between the two. Further rearings are necessary to settle this point. The mature maggots of this species almost invariably issued from their host while it was still a larva and from one to three days after its death, leaving only an empty head shell and a shriveled skin. The adult flies appeared from 11 to 19 (average 13.5) days after pupation. The eggs are white and conspicuous and are attached to any part of the host, most frequently about the thoracic segments. As many as 14 eggs have been seen on one individual, but it seems very unusual for more than one of the parasites to reach maturity, at least on a host of this size. In the one such case observed both of the two flies appearing were very small and one failed properly to expand. In fact, this fly does not seem to have the vigor of *Exorista nigripalpis*, for, although reared under exactly similar conditions, nearly half of them failed properly to expand their wings upon emergence, and some failed even to free themselves from their puparia. Several of the crambid larvæ which yielded these flies did not have eggs on them when collected, but the shells had probably been molted off after maggots had hatched and entered their host.

Only a single fly of the species *Exorista nigripalpis* Towns. was reared from a larva previously determined as *mutabilis*, and as a considerable number were obtained from larvæ known to be *trisectus* this parasite is discussed in a forthcoming paper dealing with *trisectus*.

The writer has records of two species of hymenopterous parasites attacking *Crambus mutabilis*.

*Apanteles crambi* Weed has been recorded from other species of the genus, and recently (10, p. 546) from *mutabilis* from South Dakota and Tennessee. When full-grown the grubs of this parasite emerge from their host and spin a mass of white cocoons near by. The host remains alive for several days, but finally dies without moving or feeding. The adult parasites emerge in six or seven days after the cocoon is formed.

*Macrocentrus crambivorus* Vier. has been reared twice from larvæ of *Crambus mutabilis* at Nashville, Tenn. In both cases the host larvæ were nearly full grown but appeared abnormally pale when collected. In the rearing box each constructed a silken case, and when this was examined a little later the larva had been replaced by a dense mass of elongate yellowish brown cocoons, 20 or 25 in number. The following day the white pupæ could be seen indistinctly through the cocoons. The parasites became adult and active within their cocoons eight or nine days later, but their actual emergence seemed to be entirely dependent on proper conditions of humidity. In one instance the adults, although active within the cocoons, did not emerge until the stopper of the vial was moistened, whereupon they all released themselves within five minutes. Other groups behaved in the same way unless they had been so long confined to their cocoons that they were unable to emerge at all. The adults are slender, yellowish brown in color, and very active. The females trail a long threadlike ovipositor behind them. In the writer's rearings all those emerging from a single mass of cocoons were of one sex. Others, also apparently of this species (determined by S. A. Rohwer as *Macrocentrus* sp.) were reared from two of the undetermined larvæ taken at La Fayette, Ind., in June, 1920. One yielded 14 males and the other 29 females.

## ARTIFICIAL CONTROL

Injury to corn by the striped sod webworm can more easily be prevented than remedied. When a field is in meadow or pasture, the moths are attracted to the low, rich portions where the grass growth is most luxuriant. If a sod field be broken and planted to corn, injury is most likely to occur in such portions. To prevent the injury the sod should be plowed as early as possible in the fall, in late July or August if possible. Land plowed after the middle of September generally shows little reduction in infestation compared with that plowed in the spring, because by September the majority of the larvæ have entered their winter quarters, where they are not seriously injured by plowing. If the sod is plowed early enough to deplete seriously the food supply of the fall generation and force the larvæ into winter quarters in an undernourished condition and incompletely protected, the method may prove somewhat beneficial.

Where lands permanently in grass, such as meadows, lawns, and parks, are heavily infested, premature drying and browning of the plants may be caused, becoming especially evident during periods of drought. Little can be done in such cases except to stimulate the growth of the grass by applying a quick-acting fertilizer, such as nitrate of soda. Fortunately, the portion of a meadow most attractive to this insect is the last to suffer from unfavorable moisture conditions and remedial treatment in such places will very seldom be required.

Although poisoned bran bait has been tested in a few instances, for the most part unsuccessfully, no opportunity has as yet presented itself to give this method a thorough trial. It may yet be found feasible for use in heavily infested grasslands.

## SUMMARY

*Crambus mutabilis* is a common species over the eastern half of the United States and as far west as Iowa, Utah, and Texas. Its food plants seem to be limited to the grass family.

It has been known to cause injury to young corn and to grasslands, but in such cases it is usually associated with some other species.

There are three generations a year in Tennessee, the third being the smallest, and gradually diminishing northward until there are only two at the northern limits of the species.

The moths are grass lovers and seek the lower and more luxuriant portions of pastures and meadows. They lay about 500 eggs, dropping them promiscuously as they fly.

The larvæ in the larger instars are distinctly striped and easily recognized. They construct tubular burrows in the earth opening at the surface and feed on near-by grasses, cutting off the leaves and dragging them into the burrows. In the fall they construct tubular nests among the grass stems, either above or partly in the ground, and pass the winter as partly grown larvæ, completing their growth in the spring.

*Crambus mutabilis* is more specialized than *C. hemiochrellus*, its nearest relative, and is evidently the terminus of one line of development in the genus.

The larvæ seem very susceptible to disease and are also frequently parasitized by both Hymenoptera and tachinids.

Control measures consist of early fall plowing of sod lands intended for corn the following year and rotation of pastures and meadows where the insect is destructive. Ordinarily natural agencies prevent its injurious increase.

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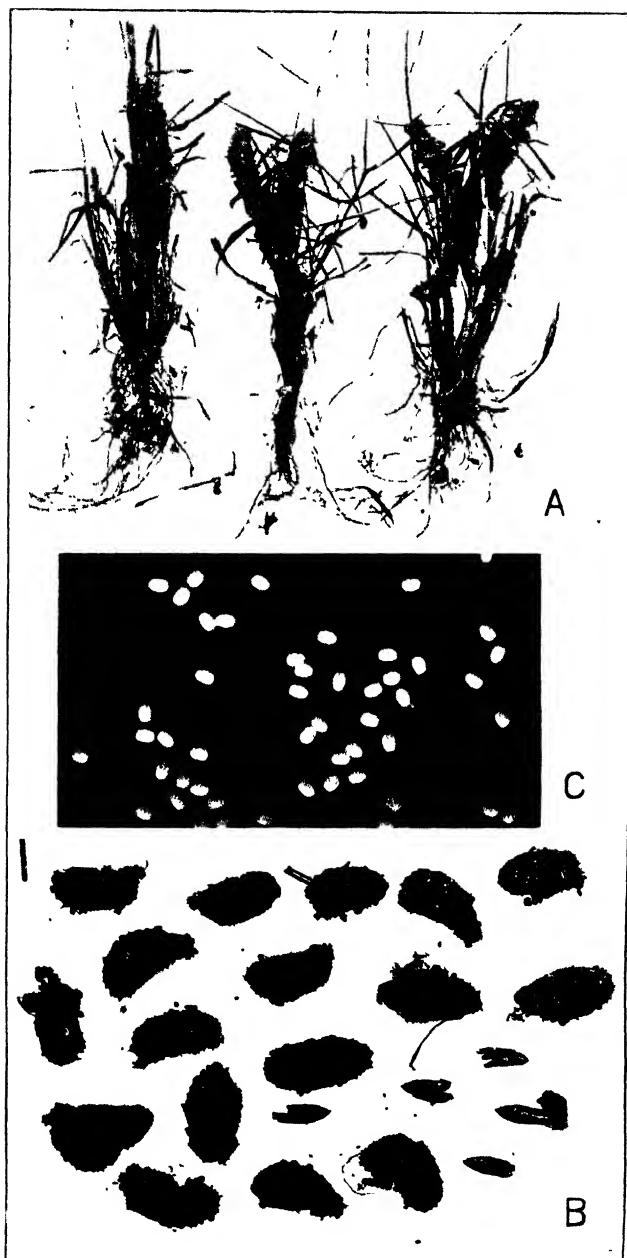




PLATE I

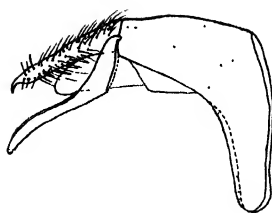
*Crambus mutabilis*:

- A.—Winter cases of larvæ on blue-grass plants.
- B.—Pupal shells and cocoons.
- C.—Eggs. Greatly enlarged.

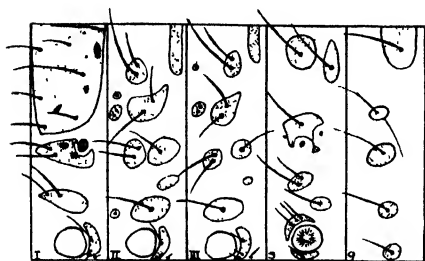




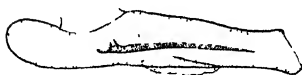
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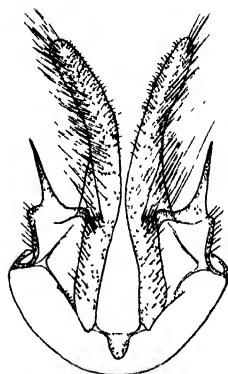
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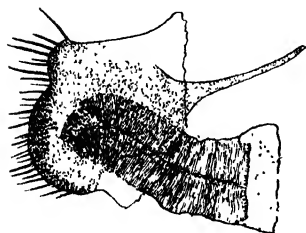
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D



F



G

PLATE 2

*Crambus mutabilis*:

- A.—Male antennal segment (twenty-fifth), greatly enlarged.
- B.—Setal map of three thoracic and third and ninth abdominal segments of larva.
- C.—Male genitalia: Tegumen and uncus.
- D.—Male genitalia: Harpes.
- E.—Male genitalia: Aedoeagus.
- F.—Female genitalia: Valve.
- G.—Tip of pupa, dorsal view.



# SILVER-STRIPED WEBWORM, CRAMBUS PRAEFECTELLUS ZINCKEN<sup>1</sup>

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## INTRODUCTION

Although of less economic importance than many other members of the genus, *Crambus praefectellus* is so widely distributed that it is sure to be met with by anyone interested in these beautiful little moths. It is one of several species with a longitudinal silvery-white stripe in the forewing (fig. 2). It is most likely to be confused with *C. leachellus* Zincken, which, however, is a larger species with the white stripe running much closer to the costal margin of the wing than it does in *praefectellus*. *Crambus quinquareatus* Zeller and *C. unistriatellus* Packard also resemble it in size and general pattern, but in the former the apex of the wing is acuminate and in the latter the white stripe runs the full length of the wing, characters which easily distinguish their possessors from the species under consideration.

## SYSTEMATIC HISTORY

*Crambus praefectellus* was first described by Zincken (9, p. 249)<sup>2</sup> in 1821 from specimens sent him from Georgia. He placed it in the genus *Chilo*, which at that time was synonymous with what we now know as the subfamily Crambinae. Clemens (3, p. 203) redescribed it in 1860 as *Crambus involutellus*. In his revision of the group in 1863, Zeller (8, p. 18) placed Zincken's species in its present genus and incorrectly placed *involutellus* as a synonym of *leachellus* Zincken. In this he was followed, with some hesitation, by Grote (7, p. 77), but Fernald (5, p. 45) corrected the error and first placed *involutellus* Clemens as a synonym of *praefectellus* Zincken, a course approved by all later writers. The synonymy then stands as follows:

*Chilo praefectellus* Zincken, 1821

*Crambus involutellus* Clemens, 1860

*Crambus praefectellus* (Zincken) Zeller, 1863

Although the bibliography of this species comprises some 25 titles, the great majority of these are merely references to the occurrence of the moths in various localities. Felt (4, p. 85) figures and discusses the species, but since he did not find it at Ithaca, N. Y., where his work was done, he gives us no biological information. Fernald (6, p. 31) figures and describes the adult and concludes with the comprehensive statement, "Early stages and food plant unknown." Since that time Britton's paper (2, p. 222) is the only publication that adds to our knowledge.

<sup>1</sup> Accepted for publication July 11, 1932. This paper is the fourth in a series of Contributions to a Knowledge of the Crambinae of North America. 1, *Crambus hemiochrellus* Zeller, appeared in *Annals of the Entomological Society of America* for March, 1918, and 11, *Crambus laqueatellus* Clemens, appeared in the June, 1931, issue of the same journal. The third paper, entitled, "Striped Sod Webworm, *Crambus mutabilis* Clemens," precedes this paper in the *Journal of Agricultural Research*.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 424-425

## GEOGRAPHICAL DISTRIBUTION

*Crambus praefectellus* is a strictly American species and seems to be limited to the eastern half of the United States. It has been taken in practically every State east of the Mississippi River and also in North Dakota, South Dakota, Minnesota, Iowa, Colorado, Missouri, Arkansas, and eastern Texas. It is reported from Cartwright, Manitoba, and also occurs in eastern Canada, at least along the southern edge. The accompanying map (Fig. 1) shows at a glance its present known distribution. The following list gives the States from which records are avail-

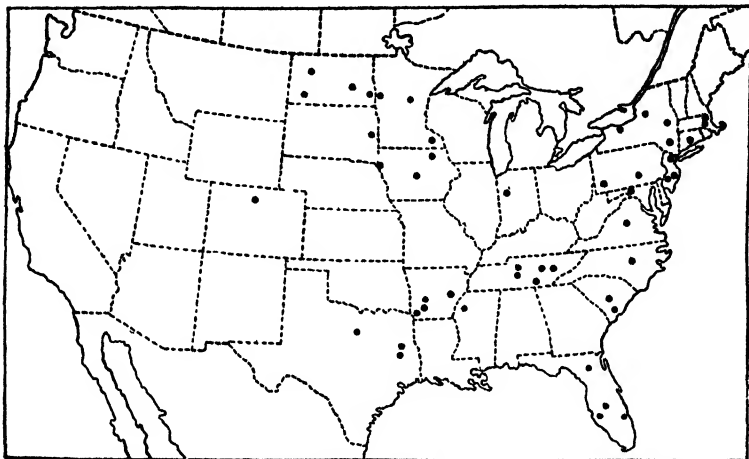


Fig. 1.—Map of the United States showing known distribution of *Crambus praefectellus*.

able and also gives the first and last date and the intervening months when collections have been made:

Arkansas. March 28, June, July 3.	New York. May 21, June, July, August 18.
Colorado. (Date uncertain.)	North Carolina. June —, July, August 23.
Connecticut. May 26, September 1.	North Dakota. June 12, July 21.
Florida. February 7, March, April 30.	Pennsylvania. May 24, June, July, August 19.
Georgia. (Date uncertain.)	South Carolina. April 1 to 7.
Illinois. May 31, June, July, August, September 3.	South Dakota. June 24.
Indiana. August 11, 12.	Tennessee. April 3, May, June, July, August, September, October 20.
Iowa. July 8, August, September 6.	Texas. January 30, March, April, May 22.
Kentucky. August.	Virginia. September 5, 6.
Maryland. August 30.	West Virginia. May 30.
Massachusetts. May 31, June, August 22.	Wisconsin. September 10.
Minnesota. June 19, July —.	Manitoba, Canada. (Date uncertain.)
Mississippi. May 3, June 22.	Ontario, Canada. June 4, July 6.
New Hampshire. August 1 to 7.	
New Jersey. May —, June, July, August, September 11.	

## FOOD PLANTS

Corn, wheat, rye, oats, blue grass (*Poa pratensis*), pigeon grass (*Setaria glauca*), and timothy (*Phleum pratense*) were all used as food plants in the writer's rearing cages and all were accepted readily by the larvæ.

Doubtless this list could be almost indefinitely extended. Judging from the habit of the moths in frequenting weedy waste ground in preference to grassy places, it is very likely that the larvæ also feed on plants other than grasses. The foregoing plants include all on which larvæ have been taken in the field.

#### ECONOMIC HISTORY

While it can not be regarded as a serious pest, *Crambus praelectellus* in one or two instances has shown that it can cause considerable injury under certain conditions. Single larvæ have been taken destroying wheat at La Fayette, Ind., and corn at Lakeland, Fla., Prescott, Brinkley, and Hot Springs, Ark., and Knoxville and Caney Spring, Tenn. Larvæ were received from Advance, Mo., with the report that they had injured 50 acres of a 300-acre cornfield. Britton's (2) recent account is the only published record of injury by these larvæ. In this case a small field of corn in the outskirts of New Haven, Conn., was almost totally ruined. The field had been in grass previously, and was plowed in the spring and planted to corn. The plants were attacked while small, and so numerous were the larvæ that only a very few plants escaped injury and produced grain.

Just what factors in the life economy of this species prevent it from more often becoming destructive can not be stated. Probably parasites, predacious enemies, and disease all play their parts, but nothing is known about these. It is apparent that this insect has not in any special way adapted its life cycle to extremes of climate. It has no definite protective resting period and consequently is overtaken by winter and unfavorable weather in all its different stages, some of them unfitted to resist such conditions. The mortality from such causes must be very great.

If control measures were needed, probably the best would be early fall plowing of land intended for corn the following season. If this were done, and the planting delayed as long as possible in the spring, the ground meanwhile being fallow and free from weeds and grass, there should be very slight possibility of the larvæ surviving until the corn germinated. If the infestation is not discovered until the corn is up, as is usually the case, little can be done but to replant alternately with the old rows, allowing them to stand as long as possible before cultivating them out. This method is described more fully in another paper (1, p. 15).

#### SEASONAL HISTORY

The earliest seasonal record for a moth of *Crambus praelectellus* is January 30, at New Caney, Tex. It has been taken at several points in Florida during February. In Tennessee, where continuous observations have been made for several years, the first moths make their appearance during April, usually toward the end of the month. It is always the first species of the genus to appear. On one occasion a battered male moth was taken at Knoxville on April 3. This is more than two weeks earlier than the moths have been taken in other years, and the pupa from which it emerged may have been formed in a particularly sheltered location.

After their first appearance the moths do not become abundant but are found singly and scatteringly throughout the greater part of the



summer. The generations are not distinct, although at some times the moths appear to average fresher than at others. It is very evident that they breed continuously and that the larvæ do not have any considerable resting period after completing their growth but pupate at once. Judging from laboratory rearing records, there are probably three generations during the year in Tennessee. Farther north this number may be lessened and at the southern limit of its range there are probably more. With a species such as this, in which the generations follow one another without intermission other than the delays due to unfavorable weather conditions, the number of generations in any given season is directly dependent on the length of the growing season and may vary from year to year.

All the available data as to the seasonal appearance of moths in other regions are so scattered and fragmentary that it seems impossible to draw any definite conclusions from them. In the list given under "Geographical distribution," the seasonal records are arranged by States. In the following list the same data are arranged by months, in order to show very incompletely the seasonal trend of the occurrence of the moths.

January.....	Texas.
February .....	Florida.
March .....	Texas, Florida, Arkansas.
April.....	Texas, Florida, South Carolina, Tennessee.
May.....	Texas, Mississippi, Tennessee, West Virginia, Illinois, New Jersey, Pennsylvania, New York, Massachusetts, Connecticut.
June .....	Mississippi, Arkansas, Tennessee, North Carolina, New Jersey, Illinois, Pennsylvania, New York, Massachusetts, Minnesota, South Dakota, North Dakota, Ontario.
July.....	Arkansas, Tennessee, North Carolina, Illinois, Pennsylvania, New Jersey, New York, Iowa, Minnesota, North Dakota, Ontario.
August .....	Tennessee, North Carolina, Kentucky, Illinois, Maryland, New Jersey, Pennsylvania, New York, New Hampshire, Massachusetts, Indiana, Iowa.
September..	Tennessee, Illinois, Virginia, Connecticut, New Jersey, Iowa, Wisconsin.
October.....	Tennessee.

#### THE MOTH

The writer has never found the moths of this species really abundant. Usually they have been taken very sparingly, one or two at a time and very seldom as many as half a dozen in a day's collecting. They were seen most abundantly at Greenwood, Miss., on the night of June 22, 1915, when 34 were taken at electric street lights between 8 and 11 p. m. In the field the moths seem to prefer more or less open, weedy or waste ground, such as neglected strawberry beds or fallow fields, rather than grassy places.

When the moths are flushed during the day, they usually fly only a short distance and may be readily captured with a small vial, but toward dusk they are much more wary, and when disturbed frequently fly 50 feet or more before settling. They alight, apparently without preference, on any part of an object, leaf, grass stem, or very frequently on the bare ground. They seldom rearrange their position after alighting. In the field, the silvery stripe and the brassy shade of the forewing in fresh specimens make them easy to identify at a considerable distance. Around lights at night they can be distinguished from *Crambus teterrellus* Zincken, the only other species of equal size with which they are apt to be asso-

ciated, by their habit of lying closely parallel with the surface on which they are at rest, quite in contrast with the moths of *teterrellus*, which rest with their heads pressed closely to the surface and their bodies elevated at an angle of 25°.

The data on hand show that these moths are not especially prolific, at least compared with some of the other species of this genus. The average number of eggs laid by the 41 females of which the writer has records was 118. This includes moths taken in the field and confined in dry vials and in tin boxes with water and with honey. The largest number laid by a single individual was 533, and only 9 of the entire 41 laid more than 200 eggs. The moths evidently mate immediately after issuing from the pupa, for not one of those collected in the field, some of them very fresh, laid infertile eggs.

There is nothing to indicate that the adults of either sex ingest anything besides water. They do not seem in the least attracted to flowers or other possible food sources. One moth excitedly waved her antennæ when approached with a droplet of honey and when finally induced to taste it, rapidly sucked it up. An attempt was made to determine if food in the form of dilute honey had any effect on the length of life or the egg production. The following table summarizes the results and leads to the conclusion that food has no pronounced effect above that of plain water, on either longevity or fecundity, but that either water or honey appreciably prolongs the life and increases egg production above that of moths confined in dry vials. As the majority of these moths were taken in the field, the averages as given are rather below than above the normal.

TABLE I.—Relation of feeding to longevity and fecundity of moths of *Crambus praefectellus*

	Male.		Female.			
	Longevity.	Number averaged.	Longevity.	Number averaged.	Eggs produced	Number averaged.
	<i>Days.</i>		<i>Days.</i>			
Water .....	9. 07	15	8. 9	8	138	8
Dilute honey .....	7. 45	20	11. 25	12	134	12
Dry .....	7. 75	4	6. 72	18	96	21

#### DESCRIPTION OF MOTH (FIG. 2)

♀ Wing expanse, 18–25 millimeters. Head, palpi, and abdomen cinereous, the abdomen lighter. Thorax and forewings golden fuscous, the latter with a silvery white stripe bordered with a fine darker line and tapering toward each end, from base to near subterminal line, a tooth near middle of lower side, and a silvery white dash above the tip and often fused with it; from this dash a dark shade with a light costal triangle above it, a light patch below it, and crossed by the plumbeous subterminal line, runs to the apex of the wing. Costal margin wider than in *leachellus*, being more than one-half the width of the white stripe at the middle of the costa. Subterminal space with 5 blackish venular dashes. Fringes white or slightly tinged with ochreous. Hind wings white or slightly cream-colored, fringes white. (Rewritten from Fernald.) The male antennæ are plainly flattened, each segment bearing a wedge-shaped process, which, in the medium segments, is provided with 8 to 10 sensoria (Pl. 1, E). The female antennæ are filiform and are beautifully banded with narrow alternate rings of brown and white (Pl. 1, F).

**GENITALIA.**—Male: Tegumen (Pl. 1, D) with body very short, about one-third the length of the limbs, which are broad, nearly straight, and almost truncate at the tip. Uncus broad at base but quickly narrowing, slender, and of uniform width for the rest of its length, the distal third dorsad thickly set with short stout spines inclined cephalad, interspersed with a few sparse hairs; gnathos glabrous, its limbs widely separated at their tips but quickly narrowing to the slender body, which considerably exceeds the uncus. Harpes (Pl. 1, B) rather narrow at base, elongate and subfalcate in general shape; costa free except at base but much modified into a short chitinated process, incurved and truncate; cucullus lightly chitinated, strongly concave, widest just above the base and narrowing gradually to the rather obtusely rounded tip, very hairy within, with an especially thick tuft just above the base. Cucullus not sharply separated from the sacculus, which is subquadrate in general outline, with a thickened costal margin and on its disk near the ventral margin a stout, heavily chitinated finger-like spine. The vinculum is much reduced and is merely a band of lightly chitinated tissue connecting the bases of the harpes. Aedoeagus (Pl. 1, C) lightly chitinated, nearly cylindrical, rounded at the base and curved in the shape of an old-fashioned pistol; at the tip truncate and somewhat bell-shaped, the internal lining for half its length roughly tuberculate; just inside the tip is a very short, sharp, chitinated thorn-like cornutus, and about two-thirds toward the base another much larger, acute, oblique spine with a very long narrow base, its tip inclined toward the tip of the aedoeagus. Anellus a mere ventral membrane. Female: Anal plate (Pl. 1, G)

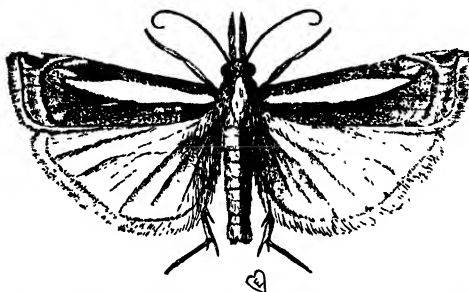


FIG. 1.—*Crambys praefecellus*: Adult. About three times natural size.

two-lobed, the dorsal lobe more feebly chitinated, about one-third the width of the lower, and separated from it by a deep notch, the margins of both lobes thickly set with stout setae.

### THE EGG

As is the case with all other species of this genus so far as known, the eggs are dropped promiscuously by the female during the early evening as she flies about or stands at rest. They are dry and drop down among the grass stems to effectual concealment in the débris beneath.

The incubation period has varied in the writer's experience from 15 days in March in Florida to 5 days in June and July in Tennessee, with all intermediate gradations. During the growing season from 5 to 9 days seems to be about the usual duration.

Almost snow white when first laid, the egg day by day becomes first pink, then flesh color, salmon, and, finally, on about the fourth day, a bright clear coral-red. They remain thus until about 24 hours before hatching, when the head and cervical plate of the contained larva begin to darken, giving the eggs a purplish hue. The larva escapes through a somewhat irregularly cut hole at one side of the larger end of the egg, leaving the empty shell nearly transparent and slightly iridescent.

The eggs of this species are somewhat rounder and with the small end a little more acute than the average for the genus. With this as with

several other species, it is found that the variations among the eggs of different individuals, especially in size, were greater than those existing between this and other species, thus rendering interspecific measurements of very little value. In fact, it holds true throughout the genus that the eggs of the various species are so similar as to render them practically indistinguishable.

To indicate the variation in size of the eggs of this species, the following measurements of two lots of 10 eggs each were made at different times:

TABLE II.—Egg measurements of *Crambus praefectellus*

Lot.	Length.			Width.		
	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
1.....	Mm. 0.5471	Mm. 0.5118	Mm. 0.5207	Mm. 0.3353	Mm. 0.3000	Mm. 0.3106
2.....	.5736	.4633	.5018	.3442	.2692	.3149

The color is of interest because it has been found that the maximum color attained during the incubation period is very similar in the various subgeneric groups of closely related species, and differs between these groups from a pale straw-yellow to a deep coral-red. Thus the egg colors as well as the head colors of the newly hatched larvæ help to indicate the affinities of the various species.

The chorion is ornamented with acute longitudinal ribs, usually 21 in number, which become obsolete before reaching the poles. The polar areas are covered with scattered oval tubercles of variable size (Pl. 1, H). Between the ribs there are also less prominent cross carinæ, about 17 of these in the length of the egg. The egg is suboval in outline, one end slightly larger and more flattened than the other.

#### THE LARVA

The writer has never taken larvæ in the field, and the only notes on their normal behavior are those contained in Britton's account (2) of the attack on corn in Connecticut. Even here the conditions were not strictly normal, for the grass sod in which the larvæ were living was plowed under in the spring and the field planted to corn, forcing the larvæ onto the young corn plants as the only available food. The larvæ fed in the manner usual to most of the species under similar conditions, cutting a hole into the tender stalk below the ground level and living in a fragile tube of silk and earth particles attached to the stalk and leading off into the ground. Except when actually at work the larva does not remain in the stalk, but in this tube, so that when the plant is pulled the author of the injury, together with most of its domicile, is likely to be left behind in the earth.

In the cages used the larvæ were reared without especial difficulty. For the most part 1-ounce or 2-ounce tin salve boxes floored with damp blotting paper were used and the food was supplied in the form of short sections of the leaves of various grasses, usually blue grass (*Poa pratensis*). Under these conditions the progress through the instars was easily watched. It was not always easy, however, to be sure that a

molt had occurred. The only sure proof was to find the cast of the head, but in this species the first act of the newly molted larva was in most cases to eat the head cast, often leaving nothing but the mandibles as evidence that ecdysis had really occurred.

The first food of the larva consists of the fragment of eggshell consumed in effecting escape from the egg. This particle becomes bright pink or salmon color in the intestinal tract. As soon as it is free from the egg the tiny caterpillar is ready for green food. When placed on a blue-grass leaf it begins operations by cutting a narrow pit lengthwise of the leaf, at first avoiding the veins. This pit soon becomes large enough to contain the entire body of its maker and then a few threads of silk are spun across above it. The excrement is placed on or among these strands and in a few hours the larva is practically concealed from sight by this filthy roof. After the first day or two the larva eats the small veins as well as the tissue between them down to the lower epidermis of the leaf, but not until it reaches the third instar does it consume the entire leaf blade. By this time it has become too large effectually to conceal itself on a blue-grass leaf, and thenceforth seeks the earth, where, for protection, it constructs a tubular retreat of mingled silk and earth particles. From this vantage ground it comes out, usually at night, to cut off and consume one by one the blue-grass leaves.

As stated above, the larvæ of this species do not spend any time in a resting period, but pupate as soon as fully fed, weather conditions permitting. Of course during the cool weather of spring and fall, and in the winter, their activities are much retarded or cease altogether, but as soon as warmer temperatures prevail feeding is resumed and the transformations completed.

The following tables give figures showing the maximum, minimum, and average periods required for the various instars and for the complete life history.

TABLE III.—Length in days of various stages and instars of *Crambus praefectellus*

A. EGGS HATCHING MAY 13

	Eggs.	I.	II	III.	IV.	V.	VI normal.	VI pre-pupal.	VII pre-pupal.	Pupa.
Maximum . . . .	9	5	5	4	6	6	6	15	8	12
Minimum . . . .	9	3	2	2	3	4	4	7	8	9
Average . . . . .	9	3.46	3.83	3.00	3.92	5.20	5.00	9.30	8.00	9.87
Number of records averaged	.....	26	29	27	27	25	2	19	1	15

B. EGGS HATCHING JUNE 25

	Eggs.	I.	II	III.	IV.	V.	VI normal.	VI pre-pupal.	VII pre-pupal.	Pupa.
Maximum . . . .	5	2	4	4	4	5	5	18	12	11
Minimum . . . .	5	2	3	3	3	2	5	11	12	10
Average . . . . .	5	2	3.50	3.17	3.82	4.25	5.00	13.14	12	10.33
Number of records averaged	.....	14	12	12	11	8	1	7	1	6

TABLE III.—Length in days of various stages and instars of *Crambus praefectellus*—Con.

C. MISCELLANEOUS RECORDS. EGGS HATCHING APRIL 21 TO AUGUST 8

	Eggs.	Larva.	Larva-pupa.	Pupa.	Entire developmental period
Maximum.....	15	38	85	18	71
Minimum.....	5	27	33	9	41
Average.....	7.73	31.5	51	11.3	51.5
Number of records averaged.....	22 lots.	14	65	21	..

In addition to those included in the tables, the writer has records of a few individuals hatching on September 18, which emerged as adults between January 23 and February 26 of the following year, after having been kept in the cool room of the insectary all winter.

It will be noted that most of these larvæ pass through six instars before pupation, but occasionally there is a seventh instar. The last instar before pupation is always much longer than those preceding, so that in order to make the averages correct these records are separated as normal and prepupal.

Through an oversight, detailed color descriptions of the various larval instars except the first were not made. The molted head casts were preserved, however, and the following list of characters is drawn up from them:

TABLE IV.—Head width of larvæ of *Crambus praefectellus*

Instar.	Minimum.	Maximum.	Average	Number of heads measured.
	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>	<i>Mm.</i>
I.....	0.229	0.229	0.229	(a)
II.....	.335	.300	.325	8
III.....	.520	.459	.512	7
IV.....	.882	.759	.826	10
V.....	1.306	.933	1.219	15
VI.....	1.493	1.306	1.399	2

\* Large number measured, but exact number not known.

In the first instar the head is shining black, paling somewhat but still fuscous in II. In III the ground color is still paler, with faintly darker areas indicating the later color pattern. In IV these markings have become much darker and more distinct, and the ground color of the head has become pale yellow. In the next two instars the colors remain the same, but become more intense. The color of the body of the larva through most of its growing period is a dull brown with a greenish tinge from the contents. The arrangement of the pinacula and setæ is shown in Plate 1, A.

## THE PUPA

The cocoon is a little case about the size and shape of a peanut meat, lined and stiffened with gray silk inside and outwardly covered with particles of earth. It generally lies so near the surface that no "neck" or emergence tube is necessary.

The pupa itself is very similar to others of this genus. Bright yellow when first formed, it soon darkens to a mahogany brown. A day or two before emergence the silvery stripe in the forewing becomes plainly visible through the covering. The

pupa is 9.0 millimeters long and 2.0 millimeters wide. The caudal process is flattened into a broadly triangular plate with sharp margins, its acute tip bent slightly ventrad. Close to the tip of this plate below are two slender bristles with upturned ends, while above, more widely spaced and standing about halfway from the tip to the basal angles of the plate, are two shorter, smaller bristles with down-turned ends. Beneath, the process is flattened but not excavated.

The data as to the duration of the pupa stage are included in Table III. It varies somewhat, depending on the temperature, in the writer's records ranging from 9 to 18 days. The last, however, is very unusual, and the average of all the records puts it at 10.65 days, which is much more nearly correct. Ten days may be taken as the usual duration of this stage during the growing season.

#### SYSTEMATIC RELATIONSHIPS

*Crambus praelectellus* is closely similar to *C. leachellus* in structure as in wing markings. The male genitalia differ in that in the former species the cucullus of the harpe is narrower and somewhat more falcate, the free costal margin is shorter and more highly chitinated, and the cornutus of the aedoeagus is smaller. *C. unistriatellus* also undoubtedly belongs to this group. Its harpes and uncus are very similar, but the aedoeagus differs somewhat in its armament. Another species, as yet not definitely determined but externally very similar to *C. leachellus*, has genitalia which place it in this group, though it is certainly specifically distinct from any of the other members. *C. quinquareatus* (considered by Felt as *C. hastiferellus* Walk.) is placed in this group by Felt (4, p. 85), but for want of more certain synonymy its position is not discussed.

#### SUMMARY

*Crambus praelectellus* is an American species widely distributed throughout the eastern half of the United States. While not often injurious, it has shown itself capable of causing serious damage.

It breeds continuously throughout the growing season. The generations are not distinct, but rearing records indicate that three generations per year is the usual number.

The moths are not often abundant. They prefer waste or weedy land and are seldom found in clean grasslands. The larvæ are readily reared on grasses. Winter is passed by the partly grown or mature larvæ. The moths from the mature larvæ emerge early in the spring and are the first *Crambus* moths to appear.

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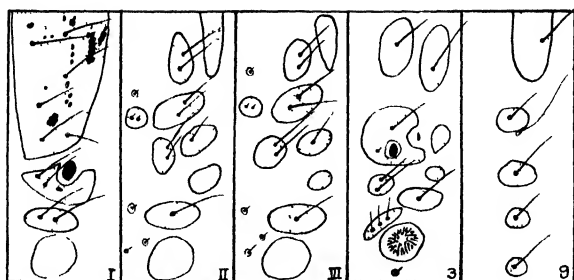
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PLATE I

*Crambus praefectellus*:

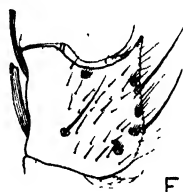
- A.—Setal map of larva showing arrangement of pinacula and setæ on the three thoracic and third and ninth abdominal segments.
- B.—Male genitalia: Harpes.
- C.—Male genitalia: Aedoeagus.
- D.—Male genitalia: Tegumen and uncus.
- E.—Male antennal segment (twenty-fifth). Greatly enlarged.
- F.—Female antennal segment (twenty-fifth). Greatly enlarged.
- G.—Female genitalia: Valve.
- H.—Polar area of egg. Greatly enlarged.



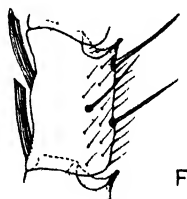
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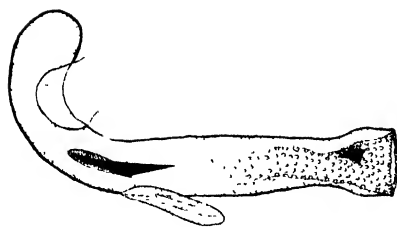
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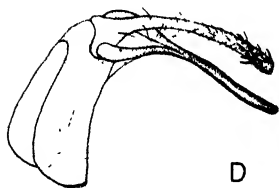
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# MOVEMENT OF SOIL MOISTURE FROM SMALL CAPILLARIES TO THE LARGE CAPILLARIES OF THE SOIL UPON FREEZING<sup>1</sup>

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## INTRODUCTION

In conducting various studies upon the temperature<sup>2</sup> and freezing-point lowering<sup>3</sup> of soils, many evidences have been obtained which indicate that the moisture of the soil upon freezing moves from the small capillaries, and probably also from around the particles as thick films, into the larger capillaries of the soil. This accumulation of the soil moisture in the large capillaries takes place especially when the moisture content of the soil is low. When the moisture content is high and under proper conditions the water accumulates as ice capillary columns at the top of the soil surfaces. These results are of considerable interest and importance as they bear on many soil-water relationships, such as the unfree water, available water, freezing-point lowering, vapor-pressure lowering, rate of evaporation, capillary movement, osmotic pressure, and perhaps others.

## EXPERIMENTAL EVIDENCES

In measuring the freezing-point lowering of soils at low moisture content, it was found that upon repeated freezing and thawing the lowering of the freezing point diminished greatly. But upon stirring the soil, even gently, the freezing-point lowering would go back to the original magnitude. If the soil was again subjected to alternate freezing and thawing, the freezing-point lowering would again diminish, and if it was stirred the lowering of the freezing point would become as before. This process could be continued almost indefinitely with practically the same results. Allowing the soil to stand after it was frozen and thawed several times tended to have somewhat the same effect as stirring, that is, it tended to restore the freezing-point depression to the original magnitude. In Table I the results in the case of two soils which will serve as typical examples of the phenomena in question are presented.

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<sup>1</sup> Accepted for publication Aug. 15, 1922.

<sup>2</sup> BOUYOUCOS, George J. SOIL TEMPERATURE. Mich. Agr. Exp. Sta. Tech. Bul. 26, 133 p. 1916.

<sup>3</sup> BOUYOUCOS, George J. and MCCOOL, M. M. FURTHER STUDIES ON THE FREEZING POINT LOWERING OF SOILS. Mich. Agr. Exp. Sta. Tech. Bul. 31, 51 p., 1 fig. 1916. Bibliographical footnotes.

TABLE I.—*Effect of alternate freezing, thawing, stirring, and standing on the freezing-point depression of soils*

## WISCONSIN SUPERIOR CLAY (20 GM. SOIL AND 4.5 CC. WATER).

Treatment.	Freezing-point depression.
	° C.
First freezing . . . . .	1.115
Second freezing . . . . .	.955
Third freezing . . . . .	.645
Fourth freezing . . . . .	.620
Stirred gently in tube with rod . . . . .	1.215
Cooled at $-10^{\circ}$ C. after stirring . . . . .	.680
Stirred gently in tube with rod . . . . .	1.215
Cooled at $-10^{\circ}$ C. after stirring . . . . .	.700
After standing one day in room temperature . . . . .	.787
Cooled at $-10^{\circ}$ C. . . . .	.701

## MICHIGAN SILT LOAM (20 GM. SOIL AND 3 CC. WATER).

First freezing . . . . .	0.880
Cooled at $-10^{\circ}$ C. . . . .	.460
Stirred gently in tube with rod . . . . .	.790
Kept at $-10^{\circ}$ for several hours after stirring . . . . .	.360
Stirred gently in tube with rod . . . . .	.785
Cooled at $-10^{\circ}$ C. after stirring . . . . .	.420
Stood in room temperature after the last reading for 10 days . . . . .	.600
Cooled at $-10^{\circ}$ C. . . . .	.360

## BLACK CLAY LOAM (20 GM. SOIL AND 4 CC. WATER).

First freezing . . . . .	0.760
Cooled at $-10^{\circ}$ . . . . .	.400
Cooled at $-10^{\circ}$ for several hours . . . . .	.270
Stood two days at room temperature . . . . .	.430
Cooled at $-10^{\circ}$ . . . . .	.322
Stirred gently in tube with rod . . . . .	.760
Cooled at $-10^{\circ}$ after stirring . . . . .	.450
Stirred gently in tube with rod . . . . .	.765

An examination of the results indicated in Table I shows the great influence that different treatments have upon the freezing-point depression of soils at low moisture contents. In the case of the Wisconsin superior clay, for instance, the freezing-point lowering diminished by alternate freezing and thawing from  $1.115^{\circ}$  to  $0.620^{\circ}$  C. at the fourth freezing. Upon stirring the soil mass with a rod in the tube, the freezing-point depression increased from  $0.620^{\circ}$  to  $1.215^{\circ}$ . When the soil was kept at a temperature of  $-10^{\circ}$  for few minutes the depression fell from  $1.215^{\circ}$  to  $0.680^{\circ}$ . As the soil was stirred again the depression rose from  $0.680^{\circ}$  to  $1.215^{\circ}$  as before. By leaving the soil to stand for one day at room temperature, after the depression was diminished by repeated freezing, the depression rose from  $0.700^{\circ}$  to  $0.787^{\circ}$ . It will be noted that in some cases stirring increases the freezing-point depression even to more than the original extent.

Results of the same type are also obtained on natural field soils. In Table II are shown the results with a silt loam taken from the field when the moisture content was moderately low and the soil had a crumb structure.

TABLE II.—*Effect of alternate freezing, thawing, stirring, and standing on the freezing-point depression of a field soil (silt loam)*

Treatment.	Freezing-point depression.
	° C.
First freezing .....	1.150
Second freezing .....	.870
Stirred gently in tube ..	1.230
Frozen once after stirring .....	.880
Cooled at $-10^{\circ}$ C. for several hours .....	.680

These results, obtained from the natural soil, agree perfectly with and confirm those from the artificially moistened soil. The question is, therefore, what factor is responsible for the great influence on the freezing-point depression of alternate freezing, thawing, stirring, and standing of the soils?

Before the effect of stirring upon the freezing-point depression was discovered, it was thought that the diminution of the lowering of the freezing point was due, at least partly, to the coagulation of the colloids upon freezing, and to the consequent liberation of unfree water from the colloids. The hypothesis<sup>4</sup> advanced was that the unfree water had a lower concentration than the free water, and upon its liberation it went to dilute the free water and thereby increased its freezing-point lowering. In view of the effect of stirring, however, this coagulation theory does not appear to afford the whole explanation for the phenomenon.

The most logical and plausible explanation that now presents itself is the assumption that, upon freezing, the moisture in the small capillaries and that surrounding the particles as thick films accumulates in the larger capillaries of the soil by the force of crystallization. In other words, the water in the larger capillaries, upon freezing, draws upon itself by the force of crystallization the water from the finer or smaller capillaries and films around the soil particles, and grows at their expense. Thus the water in the large capillaries affects the freezing-point depression differently from that in the small capillaries. How this is accomplished will be discussed later. Meanwhile, further evidence is here offered indicating that water moves from the small to the large capillaries upon freezing.

When a soil with low moisture content is frozen, small droplets or particles of ice are formed at different places in the soil mass. These ice particles or droplets occur in soils both under laboratory and field conditions and can be seen very readily and distinctly even with the naked eye. Upon close examination it is found that they occur mainly in the most porous places or in the largest capillary spaces between the soil particles.

However, when the soil is very moist and the freezing process is not too rapid, the moisture freezes at the surface of the soil in the form of

<sup>4</sup> BOUYOUKOS, George J. THE CONCENTRATION OF THE SOIL SOLUTION AROUND THE SOIL PARTICLES. *In* Soil Sci., v. 21, p. 132-138. 1921.

ice capillary columns, or long needle-like crystals. The force of crystallization seems to pull the water from below and bring it to the surface, where it freezes into these massive ice capillary columns or compact needle-like crystals. In Plate I a typical example of this phenomenon is shown. This picture was taken on a muck soil during the latter part of November, when the soil temperature below the surface was still considerably above the freezing point. The ice capillary columns would be formed at the surface of the soil without penetrating the lower depths, growing upward as straight needles or thin capillary tubes massed together. The growth seems to take place at the lower end and push the entire column upward, as the capillary tubes are elongated from below. The ice column shown in Plate I is about 4 inches thick, and was formed during three nights. The formation for each night is indicated by the lines or layers seen in the column.

As previously stated, the water which went to make this 4-inch column of ice came from the capillary water of the soil at a lower depth, and was brought to the surface by the pull or force of crystallization. From these results it is easily understood that it is possible for the moisture to move from the finer capillaries, and from around the particles as films, to the larger capillaries of a soil short of saturation. This phenomenon of the transference of moisture from the smaller to the larger capillaries upon freezing is somewhat analogous to another phenomenon—the tendency of small drops of liquid to unite into a single drop, which is accomplished either by actual contact or by the transference of vapor from the smaller to the larger drops.

In referring again to the effect on the freezing-point depression, another question arises: Why should the water in the large capillaries affect the freezing-point depression differently from that in the finer capillaries?

These differences can be easily explained if the hypothesis<sup>5</sup> previously advanced is correct. This hypothesis assumes that the solution immediately around the soil particles and in the very fine capillary spaces is less concentrated than the mass of the solution. This assumption which accords with the results presented in this paper, holds that the force of crystallization tends to draw the moisture from the finer capillaries and from around the particles as films into the larger capillaries. It is readily seen that during freezing and thawing the dilute solution from the finer capillaries and the films from around the particles go to dilute the solution in the larger capillaries or the mass of the solution. The consequence is that the original freezing-point depression is diminished. When the soil mass is stirred the moisture is again redistributed and readjusted and the freezing-point depression becomes as before.

If the hypothesis of the difference in concentration between solution in mass and that in the finer capillaries and around the soil particles is true, the above explanation is probably the correct one. But Parker<sup>6</sup> has published results to show that when the water is reduced to film or capillary form it has a decided physical effect upon the freezing-point depression. If his claim is true the explanation that immediately suggests itself is that the water in the larger capillaries has less physical

<sup>5</sup> BOUYOUCOS, George J. *OP CIT.*

<sup>6</sup> PARKER, F. W. *METHODS OF STUDYING THE CONCENTRATION AND COMPOSITION OF THE SOIL SOLUTION.* In *Soil Sci.*, v. 12, p. 209-212. 1921. References, p. 231-232.

effect upon the freezing-point depression than that in the finer capillaries and around the particles as thin films.

It can not be definitely stated now which of these two explanations is correct. If the latter one is true the position or relative distribution of the capillary water as between the finer and larger capillaries affects not only the freezing-point depression but also such factors as osmotic pressure, vapor pressure lowering, rate of evaporation, available water, etc.

Since any treatment of the soil (stirring, breaking up the compound particles or crumbs, freezing and thawing, addition of flocculent or deflocculent agents) would alter the position of the capillary water as between the small and large capillaries and films, and would have a pronounced effect upon the above factors, their determination could not be absolute. Such determinations, for instance, as the vapor-pressure lowering of soils as reported recently by Thomas,<sup>7</sup> could not be considered absolute. If stirring, freezing, etc., affect the freezing-point depression, they will certainly also affect the vapor pressure lowering. The same would be true for several of the other factors.

#### SUMMARY

Evidence is presented which shows that when a soil short of saturation is frozen, the force of crystallization tends to draw the moisture from the small capillaries and from around the particles as thick films, into the larger capillaries.

However, when the soil is wet or saturated, under proper conditions the moisture freezes at the surface of the soil and forms capillary ice columns or thin needle-like crystals. The force of crystallization draws the water from below, which freezes at the lower end of the column and pushes the entire column upward.

The relative distribution of the capillary water is between the finer and the larger capillaries and may have a very appreciable effect upon such factors as freezing-point depression, vapor-pressure lowering, osmotic pressure, and rate of evaporation. Any treatment of the soil which will alter the relative distribution of the soil moisture as between the finer and larger capillaries would seem to affect these factors.

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<sup>7</sup> THOMAS, Moyer D. AQUEOUS VAPOR PRESSURE OF SOILS. *In* Soil Sci., v. 11, p. 409-434, 5 fig. 1921. References, p. 433-434.



#### PLATE 1

Column of ice composed of thin capillary tubes or needlelike crystals formed at the surface of a wet muck. The force of crystallization brings the capillary water to the surface and, as it freezes into these massive capillary tubes, the whole column is pushed upward as the growth takes place at the lower end of the whole.

(432)





## NUTRITIVE VALUE OF THE GEORGIA VELVET BEAN (STIZILOBIUM DEERINGIANUM)<sup>1</sup>

By J. W. READ, *Head of Department of Agricultural Chemistry*, and BARNETT SURE,  
*Associate Professor of Agricultural Chemistry, Arkansas Agricultural College*

This paper is the third (6,5)<sup>2</sup> of a series of investigations covering the nutritive value of the Georgia velvet bean as originally planned and outlined by the senior author. It discusses the supplementary relationship of whole and skimmed milk to the hulled seed and the whole plant, and of the leaf and the hulls to the seed. Subsequent papers will deal with the biological analysis of velvet bean meal (ground pods and beans), the dietary deficiencies of some practical rations including the velvet bean as a certain portion of the diet, and the biological evaluation of the whole plant. These investigations are in progress.

In the authors' first paper (6) it was shown that the seed of the Georgia velvet bean, unlike most seeds so far studied, has a great abundance of vitamin A, but is deficient in salts, in quality of protein, and in vitamin B; that the raw mature seed is toxic to rats, and that the autoclaved seed, when it is the sole source of food, is inadequate even for maintenance. It was also shown that a ration composed of 60 per cent cooked seed (tough seed coats excluded) and 40 per cent dextrin served for maintenance for eight weeks. During the first six weeks all of the animals were apparently in an excellent state of nutrition, but immediately following this period their coats became rough, with some loss of hair. It was evident that a dangerous point in the maintenance curve had been reached and the ration was changed by the addition of a liberal supply of whole milk. On the modified ration all of the animals made even better than normal growth and three generations were reared successfully. Since the authors' previous work indicated that the seed is rich in the A vitamin, experiments employing skimmed milk instead of whole milk and replacing dextrin by starch were also introduced.

Recently Mattill and Conklin published a paper (2) which showed that milk, even when given in the dried form to furnish enough of the solids and fortified with iron citrate, permits no rearing of the young, although it does promote considerable growth. On a ration composed of 99 parts dried milk and 1 part yeast they secured normal growth and partially successful reproduction. For this reason, these authors suggest the possibility of yeast supplying something unique in the ration. On the basis of the results secured on their various milk diet, they also express the opinion that milk may be both quantitatively and qualitatively inadequate for adolescent growth and reproduction, especially in the female, and that it may even contain substances inhibitory to growth in

<sup>1</sup> Accepted for publication Aug. 27, 1922. Published by courtesy of the American Chemical Society; paper read at New York meeting, September, 1921.

<sup>2</sup> Reference is made made by number (italic) to "Literature cited," p. 440.

the third or mature growth cycle. In following up this work, Mattill (1) has recently reported that dilution of whole milk powder with lard, starch, and salts in varying proportions did not prevent failure of adolescent growth and reproductive ability in female rats. If, however, a small amount of yeast was added to the milk rations the females cast litters regularly and repeatedly, but the young soon died.

Since the completion of our experiments, a paper by Sherman, Rouse, Allen, and Woods (4) has appeared. Using the rat as the experimental animal, they secured practically the normal rate of growth for both sexes on a mixture of equal weights of bread and milk in which white bread furnished four-fifths and milk only one-fifth of the total calories (or a corresponding mixture of dry bread or flour and whole milk powder), but reproduction failed on this simple diet. If ground whole wheat instead of white bread or patent flour furnished four-fifths of the calories in the above ration, young were successfully suckled, though at a considerable loss of weight on the part of the mother, grew to maturity at somewhat less than the average rate, and in several cases have produced and successfully suckled young of the third generation. When the proportion of milk in the diet constituted about two-fifths of the total calories of

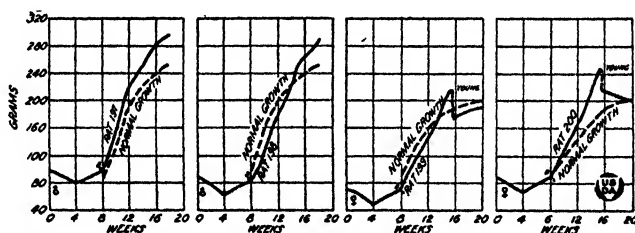


FIG. 1.—Velvet bean, 60 per cent; dextrin, 40 per cent. At point *a* a liberal supply of whole milk was added to the ration. Dotted lines represent normal curves of growth. Y=young.

the food mixture, the rest of which was ground whole wheat, the young were suckled without undue loss of the mother's weight, and these young have grown normally, as have also the young of the third generation. The inference to be drawn from their experiments is that wheat contains some substance or substances deficient in milk which are necessary for reproduction.

In the authors' experiments to determine the supplementary value of hulls (by which is meant the tough outer seed coat) to the seed, these hulls were dried and incorporated into the seed portion of the rations in the same amount as they form a natural part of the seed. The ground seed (hulls excluded) was autoclaved before feeding, but the leaf and hulls were added to the diets in their natural state.

EXPERIMENT I, LOT L.—(Charted in fig. 1.) This ration was started with 60 per cent cooked velvet bean seed (hulled) and 40 per cent dextrin. During the first six weeks of experimentation, the animals were all in a perfect maintenance condition, after which period they began to show characteristic signs of malnutrition, as evidenced by the roughness of their coat, lack of energy, loss of hair, etc. At point *a* a liberal supply of whole milk was added to the ration. From that period a little better than normal growth was obtained. Rat 200 gave birth to eight young

but was allowed only four to rear, in order not to overtax her mammary capacity. These four young were successfully reared to weaning age.

EXPERIMENT II, LOT L.—(Charted in fig. 2.) This experiment shows the successful normal growth of the young of rat 200 to maturity. These animals were permitted to interbreed, and it will be noted that rat 471 reared the third generation successfully. Rat 200 was then bred to one of her sons and successfully reared all the eight young which she brought forth at birth. (Not shown on fig. 2.) Our efforts to substitute the uncooked seed for the cooked met with failure, due evidently to the toxic principle in the raw seed.

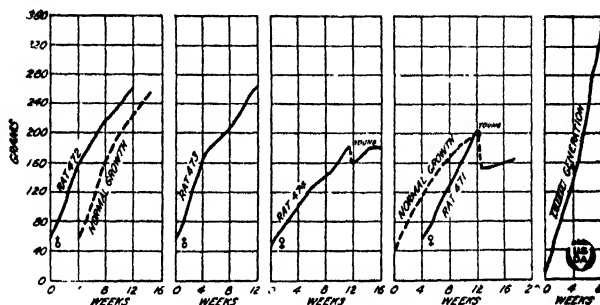


FIG. 2.—Second generation Velvet bean, 60 per cent, dextrin, 40 per cent, plus a liberal supply of whole milk. Dotted lines represent normal curves of growth. Y=young.

EXPERIMENT III, LOT CXIII.—(Charted in fig. 3.) On a ration composed of 40 per cent velvet bean hay (finely ground whole plant), 60 per cent starch, and a liberal supply of skimmed milk, rat 454 successfully reared the four young to which she gave birth.

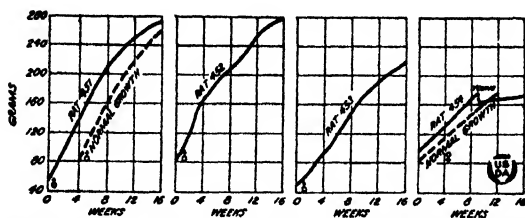


FIG. 3.—Velvet bean (whole plant), 40 per cent, starch, 60 per cent; and a liberal supply of skimmed milk. Dotted lines represent normal curves of growth. Y=young.

EXPERIMENTS IV, V, LOT CXIII.—(Charted in fig. 4 and 5.) This experiment showed that quite satisfactory growth was secured through the second and third generations on this simple and monotonous ration of whole plant 40, starch 60, and a liberal supply of skimmed milk. We attribute the poorer showing of the animals on Figure 4 to hot weather and less consumption of milk due to rapid souring. The whole plant and starch mixture was readily eaten by all of our animals on this ration.

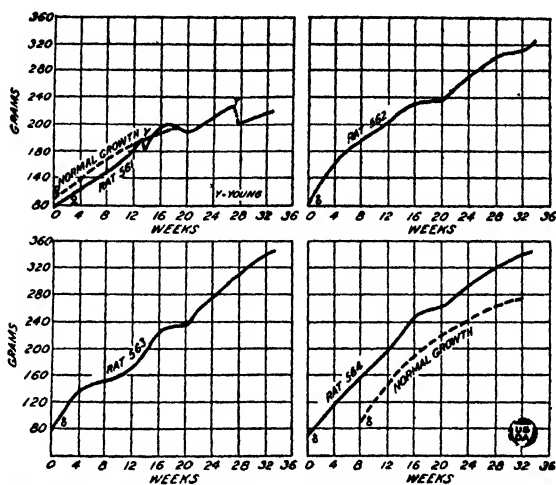


FIG. 4.—Second generation. Velvet bean (whole plant), 40 per cent; starch, 60 per cent, and a liberal supply of skimmed milk. Dotted lines represent normal curves of growth. Y=young.

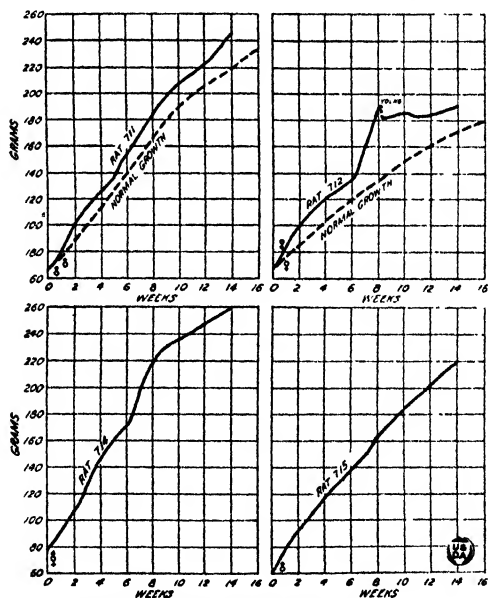


FIG. 5.—Third generation. Velvet bean (whole plant), 40 per cent; starch, 60 per cent, and a liberal supply of skimmed milk. Dotted lines represent normal curves of growth.

**EXPERIMENT VI, Lot LXXVIII.**—(Charted in fig. 6.) This experiment showed that the velvet-bean hulls offer no supplementing value to the deficient proteins in the seed, neither was there any appreciable change in the character of growth when, at point *a*, 21 per cent of dextrin was replaced by 21 per cent velvet-bean leaves.

**EXPERIMENT VII, Lot LXXVI.**—(Charted in fig. 7.) It is apparent from this experiment that the hulls in the velvet bean, unlike those in

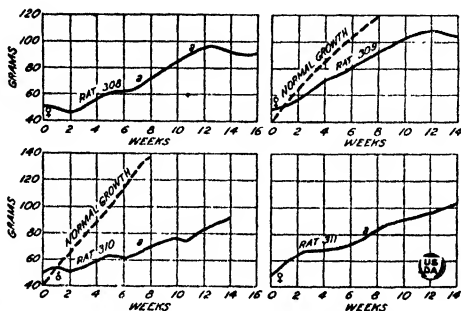


FIG. 6.—Velvet bean, 53 per cent, hulls, 7 per cent, salts (No. 32), 4 per cent, butter fat, 5 per cent, dextrin, 21 per cent. Dextrin carried alcoholic extract of 10 grams ether-extracted wheat embryo. At point *a* the 21 per cent dextrin was replaced by 21 per cent velvet bean leaves to furnish protein. Dotted lines represent normal curves of growth.

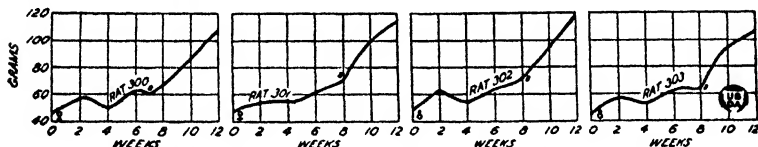


FIG. 7.—Velvet bean, 53 per cent, hulls, 7 per cent, salts (No. 32), 4 per cent, butter fat, 5 per cent, casein, 5 per cent, dextrin, 26 per cent. At point *a* the 26 per cent dextrin was substituted by 26 per cent velvet-bean leaves to furnish the water-soluble vitamins.

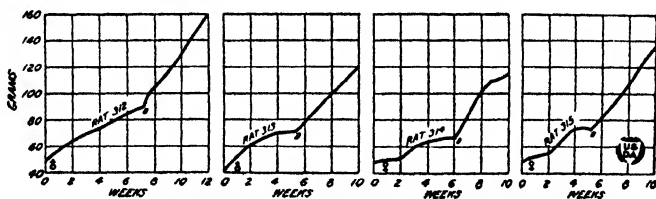


FIG. 8.—Velvet bean, 53 per cent, hulls, 7 per cent, butter fat, 5 per cent, casein, 5 per cent, dextrin, 30 per cent. At point *a* 10 per cent dextrin was replaced by 10 per cent velvet bean leaves to furnish salts. Dextrin carried alcoholic extract of 10 grams ether-extracted wheat embryo.

the rice kernel, are not carriers of the B vitamins. When, however, at point *a* 26 per cent dextrin was replaced by an equivalent amount of velvet-bean leaf a striking change in the character of growth was obtained in all cases.

**EXPERIMENT VIII, Lot LXXIX.**—(Charted in fig. 8.) The hulls offer very little supplementing value to the seed in so far as salts are concerned, but as low as a 10 per cent concentration of velvet-bean leaves furnishes a very satisfactory source of salts when added to 53 per cent of the seed.



EXPERIMENT IX, LOT LXVII.—(Charted in fig. 9.) This experiment showed that when the velvet-bean seed serves as the only source of the A vitamine fed at a 60 per cent level very good growth is secured.

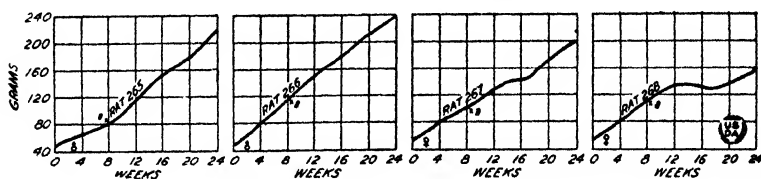


FIG. 9.—Velvet bean, 60 per cent, casein, 5 per cent, salts (No. 32), 4 per cent; dextrin, 31 per cent. Dextrin carried alcoholic extract of 10 grams ether-extracted embryo. At point a 4 per cent dextrin was replaced by 4 per cent additional casein.

EXPERIMENT X, LOT LXXX.—(Charted in fig. 10.) It is quite apparent from this experiment that considerable inferior growth is obtained when 7 per cent of hulls is added to a ration containing 60 per cent of the hulled seed, 7 per cent being the concentration of hulls in the whole seed.

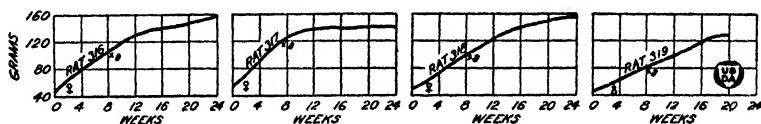


FIG. 10.—Velvet bean, 53 per cent, hulls, 7 per cent; casein, 5 per cent, salts (No. 32), 4 per cent, dextrin, 32 per cent. Dextrin carried alcoholic extract of 10 grams ether-extracted wheat embryo. At point a 4 per cent dextrin was replaced by 4 per cent additional casein.

EXPERIMENT XI, LOT CXXIX.—(Charted in fig. 11.) This experiment shows that autoclaving the hulls at 15 pounds pressure for two hours does not remove any apparent toxic substance, and gives additional evidence that the hulls interfere with the utilization of vitamine A.

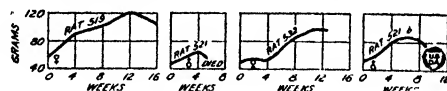


FIG. 11.—Velvet bean, 53 per cent, hulls, 7 per cent, casein, 9 per cent, salts (No. 32), 4 per cent; dextrin, 27 per cent. The hulls in this ration were autoclaved for two hours at 15 pounds pressure. Dextrin carried alcoholic extract of 10 grams ether-extracted wheat embryo.

## DISCUSSION

The data for milk presented in this paper are in harmony with the results obtained by Mattill and Conklin (2), which show that milk alone, while it allows a certain amount of growth to take place, is inadequate for reproduction in the albino rat. The attempts made by the authors to furnish liquid milk and dextrin with a liberal supply of distilled water resulted in complete failure in most cases, and in only a few cases was some growth obtained. It is quite evident then that milk is lacking in one or more dietary essentials indispensable for satisfactory growth and for reproduction.

From a consideration of the various experiments on nutritive value of milk (reported in the literature) and in so far as Osborne and Mendel (3) have shown that the amino-acid requirements for growth and for maintenance are different, it seems to the authors that the difficulty may possibly be found with the milk proteins.

It has already been stated that Sherman, Rouse, Allen, and Woods (4) found that wheat in certain proportions supplemented milk to the extent of enabling mother rats to rear their young successfully, and on these diets they secured normal nutrition through three generations. The authors found in an extensive study of the nutritive value of the Georgia velvet bean that the velvet bean seed, a legume, supplements whole milk to the extent that three generations have been secured, all of the animals having performed even a little better than normal in their rate of growth. Even on such a simple and poorly constituted physical diet as that composed of 40 per cent velvet bean hay, 60 per cent starch, and a liberal supply of skimmed milk, three generations have been successfully produced by the authors.

The leaf of the Georgia velvet bean is an efficient carrier of salts and vitamine B. Portions of the leaf (10 and 26 per cent) added to 53 per cent of the seed improved the nature of growth considerably from the standpoint of both salts and the B vitamine, respectively. The hulls, however, seem to have no biological value. The authors' results show, however, that they interfere with the utilization of the A vitamine when added to a 60 per cent intake of the seed in the same proportion in which they occur in the seed. It might be argued that the inferior growth obtained by the addition of 7 per cent hulls could be due to a reduction of the total plane of intake from 60 to 53 per cent. However, such is not the case, because comparable growth was obtained when only 20 per cent of the seed, without the hulls, served as the only source of vitamine A.

The composition of velvet bean hulls, as reported by Tracy and Coe (7, p. 31), is as follows:

	Per cent
Ash.....	6.0
Crude protein.....	5.7
Fiber.....	30.0
Nitrogen-free extract.....	57.0
Fat.....	1.1

It will be noted from the above table that the hulls are very abundant in fiber, or indigestible celluloses. It seems reasonable, therefore, to suggest that the interference of the hulls with the utilization of vitamine A may be attributed to their indigestible celluloses. Autoclaving the hulls for two hours at 15 pounds pressure did not change the nature of their disturbing effect.

#### SUMMARY

(1) The velvet-bean seed (cooked) when fed at a level of 60 per cent together with 40 per cent dextrin, and velvet-bean hay (whole plant) when fed at a 40 per cent plane of intake together with 60 per cent starch, supplement milk in a manner satisfactory for growth and reproduction.

(2) The Georgia velvet-bean leaf is quite abundant in the B vitamine and contains salts of excellent biological value.

(3) The hulls have no supplementary value, and interfere with the utilization of the A vitamine in the seed. Autoclaving for two hours at 15 pounds pressure did not change their disturbing effect.

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No. 6

## SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEET POTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS<sup>1</sup>

By J. I. LAURITZEN and L. L. HARTER

*Pathologists, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

### INTRODUCTION

It has been shown (6)<sup>2</sup> that the following species of *Rhizopus* may decay sweet potatoes: *Rhizopus nigricans*, Ehrenb., *R. reflexus* Bainier, *R. artocarpi* Racib., *R. delemar* (Boid) Wehmer and Hanzawa, *R. oryzae* Went. and Pr. Geerligs., *R. tritici* Saito, *R. nodosus* Namysl., *R. arrhizus* Fischer, and *R. maydis* Bruderl. Infection was accomplished by introducing 24- to 48-hour-old cultures grown on sweet potato decoction into "wells" (5) made in the sweet potatoes, the "wells" being sealed over with a cover slip set in vaseline. The potatoes were then placed in moist chambers and incubated at temperatures suitable for infection by the species under investigation. Whether or not the capacity on the part of these species to decay sweet potatoes by the above-mentioned method is an indication of the species involved in the decay of sweet potatoes in the storage house at the Government experimental farm at Arlington, Va., and at different temperatures in infection chambers, will be answered, in part at least, in this paper.

The writers<sup>3</sup> have found, by employing the "well" method of inoculation, that the temperature range at which the parasitic species will infect and decay sweet potatoes is nearly as wide as their temperature growth range in artificial cultures. By this method the species employed have an unusual opportunity to cause infection, even to the exclusion of any other species<sup>4</sup> that might be present in the "wells" or on the surface of the potatoes. It would be difficult for any other species present in the "well" to make any headway against the mass of mycelium of a 48-hour-old culture. What might take place under storage conditions may be a different question. Here the inoculum is limited, the species present are in competition with each other, and all the species may not be present. An effort was made, therefore, to determine whether or not the parasitic species infect sweet potatoes over their entire temperature growth ranges, where infection depends upon wounding and the species present on the potatoes.

Hanzawa (2) arranged the species studied by him into three groups according to their temperature relations. There was, as is shown by his

<sup>1</sup> Accepted for publication Aug. 25, 1922.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 456.

<sup>3</sup> This statement is based on unpublished data.

<sup>4</sup> There are nearly always some *Rhizopus* spores on the surface of sweet potatoes and, since the only precaution observed to eliminate them in these experiments was by washing the potatoes, there probably were occasional spores present.

data, considerable overlapping of the temperature range of one group onto that of another. If spores of all the species were present on the sweet potatoes, one might expect the species found within one of these groups to predominate to a greater or less degree in the amount of infection they provided, within the temperature ranges of the particular group, unless the number of spores of some of the species present that belonged to the other groups greatly exceeded that of the particular group under consideration. Within the limits of their temperature ranges one would expect to find some infection by all the species present. The results of the investigation in this field will be discussed later in detail.

### APPARATUS

Much of the investigational work was carried out in a series of insulated infection chambers, the construction and dimensions of which are given in figure 1. Heat is supplied by means of a heating coil composed of

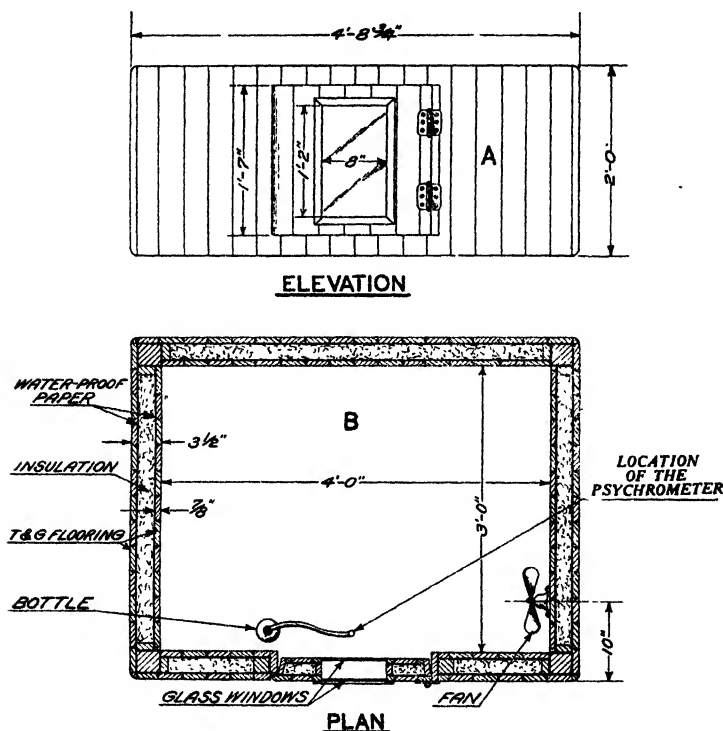


Fig. 1.—Infection chamber. A, front view; B, horizontal cross section.

No. 27 nichrome wire wound around 3/8-inch transite board 2 inches wide and 14 inches long. The floor and end wall immediately adjacent to the heating coils are insulated with transite board. Evaporating pans for the control of the humidity are located one-half inch above the heating coils. Each chamber is provided with standardized dry and wet bulb thermometers, the latter being covered with thin muslin kept wet by a capillary stream of distilled water, the excess of which is drained off by

means of a funnel into a pan located in the door of the chamber. A mercury thermoregulator, which is connected to the heating coils and a 250-ohm relay on a nearby switchboard, is suspended by a rubber band on the back wall. The air within the chamber is kept in motion by means of an 8-inch fan run by a motor located just outside the chamber. The thermoregulator is similar to that designed by Clark (1), but differs from it in that there are three arms of mercury instead of one and that one of the electric connections is made directly to one of these arms instead of through a special tube.

The storage house was constructed according to Government plans (7). It is provided with two stoves, one at each end, to furnish heat during the curing period, and with steam radiators, one flanking each corner, for the control of the temperature during the winter.

#### CONTROL OF ATMOSPHERIC ENVIRONMENT IN THE INFECTION CHAMBERS

##### TEMPERATURE

The thermoregulator used in the chambers controls the temperature within one-half of one degree. The fineness of the control will depend upon the size of the mercury column in the capillary tubing, the amount of mercury used, and its surface area.

The amount of current that passes through the heaters materially affects the control of temperature. Two amperes with a voltage of 110 is all that a 250-ohm relay will handle; in fact, sticking of the relay may be expected at times with this amount. If more current is desired a larger relay should be employed. An ampere and a half is the most that is used in any of the chambers.

To further reduce the danger of sticking, a condenser of  $1\frac{1}{2}$  microfarad capacity is employed in connection with each relay. With this type of equipment 20 chambers have been run for 9 months without a single case of sticking.

The capacity of the heaters should be limited to the amount of heat required to heat the chamber to within a few degrees above the desired temperature, otherwise there is a tendency for the temperature to fluctuate or lag.

The amount of current that flows through the thermoregulator should be reduced to a minimum consistent with efficient operation, otherwise the mercury and contact points become insulated and prevent efficient operation.

Direct current is preferable, particularly where mercury thermoregulators are employed. Current with a low number of cycles should not be used when avoidable, since the vibration set up by such a current does not contribute to effective control.

The contact points on the relays should be kept tight, in position, and clean; otherwise they become insulated by the finely divided metal thrown off, especially where a considerable amount of current is used, and thereby reduce the current below that actually required to maintain the desired temperature.

A uniform temperature throughout each chamber was obtained by the use of an 8-inch fan running at about 700 revolutions per minute.

The temperature of the air surrounding a chamber was below that of the chamber, and the desired temperature obtained by heating. The lower temperatures were obtained by placing the chambers in cold storage rooms where the temperature had been reduced by refrigeration.

## HUMIDITY

A high relative humidity (about 95 per cent), maintained by means of distilled water in evaporation pans, was used throughout these experiments. The uniformity of the humidity throughout the chambers was maintained by the circulation of the air by means of a fan. In the absence of air circulation there would be, undoubtedly, a higher relative humidity in some parts of the chambers than in others, depending on the vapor pressure in the particular area.

## GASES

In order to eliminate any possible effects of gases given off by the sweet potato or other vegetables, provision was made for a constant exchange of air, to be drawn through the chambers slowly by means of a vacuum pump. The amount of air drawn out was roughly determined by drawing it through wash bottles and regulated by stop-cocks.

## MATERIALS

The Little Stem Jersey variety of sweet potatoes grown and stored at the Government experimental farm at Arlington, Va., was employed in these experiments. The potatoes were cured for a period of 10 days at temperatures from 25° to 30° C., and held as nearly as possible throughout the season at temperatures between 10° and 14°.

The following species of *Rhizopus* were employed: *R. nigricans*, *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. arlicarpi*. They were grown on sweet potato agar at a temperature of 20° C.

## EXPERIMENTAL DATA

Four types of experiments were used. The methods employed, the purpose of the experiments, and the data obtained will be discussed in connection with each type of experiment.

## TYPE ONE

In the first type of experiments the potatoes were merely wounded and placed in the storage house at the Government experimental farm at Arlington, Va., and at different temperatures in the infection chambers. The potatoes were wounded by striking them three or four times on the blunt rim of a wire basket, after which they were placed in wire baskets 12 inches deep and 12 inches in diameter, which were in turn placed in the sweet potato storage house or in the infection chambers.

The purpose of these experiments was to determine what organisms cause decay where infection depends upon the organisms present on the potatoes. The scope of the experiments in this connection was limited to potatoes grown and stored at Washington, D. C. The number of species and the number of spores of each species present on the potatoes might be expected to vary with the locality in which the potatoes were grown, the conditions under which they were stored, and the conditions prevailing in the railway car during transit to the markets and in the markets themselves. The number of spores of a given species also might be expected to vary with the season of the year; in fact, it has been shown that there are fewer spores on the potatoes at digging time and

during the early part of the season than later in the year. There are, however, a sufficient number of *Rhizopus* spores present during the early part of the season to cause a large percentage of infection when the potatoes are wounded by the method mentioned above.

Fresh wounding contributes materially to infection by *Rhizopus*.<sup>5</sup>

It has been found, as will be shown later in this paper, that fresh wounding is all that is required to permit of infection under the conditions of these experiments.

Table I contains the results of isolations from potatoes that were wounded as mentioned above, but not inoculated. Inspection of the table reveals that two species, *R. nigricans* and *R. tritici*,<sup>6</sup> are probably the species chiefly responsible for the decay of sweet potatoes. The absence of the other species would explain why there was no infection by them, but it is not known whether or not they were present. The presence of *R. tritici* and *R. nigricans* in greater quantity (either as to spores or mycelium) than the other species might explain these results. Later data will indicate to some degree how far this is the case with *R. nigricans*.

TABLE I.—Isolations<sup>1</sup> from uninoculated wounded sweet potatoes held at different temperatures

Temperatures. °C	Organisms isolated.	
	<i>Rhizopus tritici</i> .	<i>Rhizopus nigricans</i> .
37. . . . .	60	..
32. . . . .	62	3
28.5 . . . .	8	3
27.5 . . . .	30	5
26.5 . . . .	21	19
25. . . . .	..	14
23 . . . . .	4	47
20 . . . . .	..	17
19 . . . . .	..	6
18 . . . . .	..	19
15 . . . . .	..	19
14 . . . . .	..	78
12 . . . . .	..	74
11 . . . . .	..	4
10 . . . . .	..	20
3-3 . . . . .	..	4 M, <sup>2</sup> 2 M. and R. n.

<sup>1</sup> The figures in the columns under the heading "Organisms isolated" represent the number of isolations of the particular organism at the various temperatures. They have no relation to the number of infections, which was always nearly 100 per cent, except between 20° and 30° C., where it was somewhat less. The figures in the succeeding tables under the above heading likewise refer only to the number of isolations.

<sup>2</sup> M.—Mucor. R. n.—*Rhizopus nigricans*.

Only *R. nigricans* was isolated at temperatures below 20° C. It will be seen, therefore, that *R. nigricans* is the species involved in the decay of sweet potatoes at the usual storage temperatures. The importance of *R. nigricans* on storage will be taken up more fully later.

<sup>5</sup> A paper on wounding as a factor in storage of sweet potatoes is in course of preparation.

<sup>6</sup> Although it is thought by the authors that *R. tritici* is the predominating species at the higher temperatures, it is impossible at present to be certain that *R. nodosus*, *R. oryzae*, and *R. delemanii* are not responsible for decay in some instances, and even to the same degree as *R. tritici*. As far as the authors are concerned, there are no characters by which these four species can be definitely separated. *R. tritici*, therefore, will be employed as the name to designate this group of species.



It is interesting to note that *R. tritici* in these experiments did not cause decay of sweet potatoes over its entire temperature range of growth. (Range of growth and infection for *R. tritici* is about 5° to 44–45° C.) Infection by *R. tritici* did not take place by the method employed here, at temperatures at 20° C. and below notwithstanding the fact that it takes place readily where the "well" method of inoculation is used.

The temperature ranges of infection by *R. tritici* and *R. nigricans* overlap between 20° and 30° C. Infection by *R. nigricans* becomes progressively less with the increase in temperature above 20°. *R. tritici* shows a similar decrease in the amount of infection as the temperature passes from 30° to 20°. Infection by the two species becomes nearly equal at 26.5° (Table III).

Results in connection with these and other experiments show that a smaller percentage of the potatoes decay at temperatures between 20° and 30° C. than at higher or lower temperatures. Decay also will start in a large number of cases, then stop. Whether or not these temperatures are more favorable for the potatoes and less favorable for the pathogens, or both, is not clear, except that temperatures near 30° are less favorable for *R. nigricans*.

Table II shows the results of isolations made from wounded but uninoculated potatoes used in experiments in the storage house and in the infection chambers at temperatures at which sweet potatoes are usually stored. The top row of figures represents the average of three experiments, run at different times of the year in the storage house. Most of the isolations proved to be *R. nigricans*. However, in two out of the three experiments, *R. tritici* was isolated in nine instances. Records show that the temperatures were above 20° C. for a few hours during the early part of these experiments. These results indicate that *R. tritici* may be a factor in sweet-potato storage if the temperatures are high enough. *R. nigricans* is, however, the principal cause of decay in the storage house. The four lower rows of figures in Table II represent the results obtained from four experiments run in the infection chambers at temperatures (10° and 14°) corresponding closely to those used in storage. *R. nigricans* was invariably isolated.

TABLE II.—Isolations from wounded uninoculated sweet potatoes held in the storage house and in the infection chambers at storage temperatures

Temperatures	Organisms isolated—Number of cultures isolated		
	<i>Rhizopus nigricans</i>	<i>Rhizopus tritici</i>	Mixed cultures of <i>Rhizopus tritici</i> and <i>Rhizopus nigricans</i> .
°C			
10–25 <sup>1</sup> . . . . .	140	9	3
14 . . . . .	78	0	0
12 . . . . .	74	0	0
11 . . . . .	4	0	0
10 . . . . .	3	0	0

<sup>1</sup> The figures 10–25 indicate that the temperature varied between 10 and 25°

## TYPE TWO

In the second type of experiments the sweet potatoes were wounded as above and dipped in a spore suspension made by introducing the spores of an equal number of cultures of *R. tritici* and *R. nigricans*, grown under identical conditions, into 10 gallons of water. From 8 to 10 cultures of each organism were usually employed.

It was discovered in the first type of experiments that *R. tritici* and *R. nigricans* were the species chiefly responsible for the softrot of sweet potatoes at Washington, D. C. *R. nigricans* was responsible for all the decay at temperatures below 20° C., quite to the exclusion of *R. tritici*, notwithstanding the fact that *R. tritici*, as shown by unpublished data, has the capacity of causing decay between 6° and 44°, where the "well" method of inoculation is used. Both *R. tritici* and *R. nigricans* decayed sweet potatoes between the temperatures of 20° and 30° and *R. tritici* above 30°. It is possible that the amount of infection by these species, as well as their temperature ranges, may have been influenced by the number of spores present on the potatoes. The number of spores present of both species, in turn, may have been influenced by local conditions and the conditions under which the potatoes were stored. The storage temperatures were nearly always below 20°, except during the curing period of from 10 days to 2 weeks. Since *R. nigricans* is essentially a low-temperature form, these temperatures are more favorable for its development than for *R. tritici*, which grows better at high temperature. The second series of experiments, therefore, were designed to determine whether or not the range of infection discovered in the first series would be altered in any way, especially the lower limit at which *R. tritici* caused infection, when the sweet potatoes were inoculated with approximately the same number of spores of each species.

Table III shows the results of 920 isolations made during two years in connection with 15 different experiments, most of which were designed to determine the distribution of decay by *R. tritici* and *R. nigricans* at different temperatures. In 12 of these experiments, wounded potatoes were inoculated with a spore suspension of *R. tritici* and *R. nigricans*. In the other 3 experiments the potatoes were wounded but not inoculated, and the data include all the isolations made from potatoes held at 14° C. and 74 out of the 81 isolated from potatoes held at 12°. Some of the experiments in which the isolations were made from potatoes held at low temperatures (7° and below), were designed to determine the lower limit at which these organisms would decay sweet potatoes. In the latter experiments the potatoes were wounded and inoculated as described in the first part of this paragraph. In these experiments the two species are distributed according to temperature, in much the same manner as shown in Table I. The upper and lower limits for infection by *R. tritici* are approximately 44° and 20°, respectively. The highest and lowest temperatures at which *R. nigricans* caused infection were 27° and 3.5°, respectively.

These results tend to indicate that the range of temperatures at which these species will infect sweet potatoes is so wide as to leave little possibility of adjusting temperatures in which to store the potatoes which will be outside the range of infection by these organisms. Although it has been found that these organisms do not cause any appreciable amount of decay at temperatures below 6° C. it was shown by Harter, Weimer, and Adams (5), and later verified by the authors, that if potatoes were

held for a few weeks below this temperature they almost invariably decayed with *Mucor racemosus* Fres. There is nearly always 100 per cent infection by *M. racemosus* under cold storage conditions and also in the infection chambers below 6°.

TABLE III.—Isolations from wounded sweet potatoes inoculated with *R. tritici* and *R. nigricans* and held at different temperatures

Temperatures.	Organisms isolated					
	<i>Rhizopus tritici</i> .	<i>Rhizopus nigricans</i> .	<i>Mucor</i> .	<i>Botrytis</i> .	Bacteria.	Mixture of organisms.
°C.						
44 . . . . .	3	...	...	...	12	19 P. and 1 bact.
40 . . . . .	10	...	...	...	...	
39 . . . . .	15	...	...	...	...	
38 . . . . .	15	...	...	...	...	
35.4 . . . . .	10	...	...	...	...	
33.5 . . . . .	39	...	...	...	...	
32.7 . . . . .	10	...	...	...	...	
31.4 . . . . .	10	...	...	...	...	
30.8 . . . . .	33	...	...	...	...	
30 . . . . .	48	...	...	...	...	
29.5 . . . . .	25	...	...	...	...	
28.9 . . . . .	5	...	...	...	...	
27 . . . . .	35	4	...	...	...	
26 . . . . .	16	14	...	...	...	
23 . . . . .	34	23	...	...	...	2 R. n. and R. t.
20 . . . . .	2	48	...	...	...	1 R. n. and R. t.
19 . . . . .	...	27	...	...	...	1 R. n. and R. t.
15 . . . . .	...	21	...	...	...	
14 . . . . .	...	78	...	...	...	
12 . . . . .	...	81	...	...	...	
11 . . . . .	...	46	...	...	...	
10 . . . . .	...	14	...	...	...	
9 . . . . .	...	19	...	...	...	
7 . . . . .	...	10	...	...	1	20 M. and R. n.
6 . . . . .	...	29	24	...	...	31 M. and R. n.
5.5 . . . . .	...	...	5	22	...	
3.5 . . . . .	...	3	15	...	...	3 M. and R. n.
2.7 . . . . .	...	...	27	...	...	
2 . . . . .	...	...	10	...	...	

1 P = Penicillium; bact. = bacteria; R. n. = *Rhizopus nigricans*; R. t. = *Rhizopus tritici*; and M = *Mucor*.

If this method of wounding is employed, whether the potatoes are inoculated or not, the percentage of infection by *R. tritici* and *R. nigricans* is nearly always 100 at temperatures above 30° and below 20° C., respectively, the percentage being lower between these temperatures.

Sweet potatoes are sometimes injured by heat at 40° C. if the humidity is high, and badly injured at 44°, the highest temperature at which *R. tritici* caused infection.

#### TYPE THREE

The third type of experiments was designed to determine how effectively other species compete with *R. nigricans* in producing infection at the usual storage temperatures. The potatoes were wounded as in previous experiments, some having been previously washed in tap water to reduce if possible the number of spores present on their surfaces and

others were inoculated while in the condition in which they came from the storage house. Washed and unwashed potatoes, wounded in the same manner as those inoculated, were used as controls in all the experiments.

The following species were employed in these experiments: *R. nigricans*, *R. reflexus*, *R. artocarp*i, *R. tritici*, and *R. oryzae*. Washed and unwashed potatoes were inoculated with each of the species alone, except *R. nigricans*. Inoculation in each case was by dipping them into a spore suspension. Washed and unwashed potatoes were inoculated with a mixed spore suspension of *R. reflexus* and *R. nigricans*. Washed potatoes were inoculated with a mixed spore suspension of *R. artocarp*i and *R. nigricans*, and unwashed potatoes with *R. tritici* and *R. nigricans*.

In the preceding experiments *R. nigricans* was shown to be the principal organism causing decay at the usual storage temperatures. This species has also been shown to cause decay of many fruits and vegetables. (4) It was found in the second type of experiments that when the sweet potatoes were inoculated with *R. tritici* and *R. nigricans*, *nigricans* caused all the decay below 20° C., notwithstanding the fact that *R. tritici* is not only capable of infecting potatoes at these temperatures, but apparently<sup>1</sup> to the same degree where the "well" method of inoculation is employed.

In view of these facts it was thought desirable to make competitive tests between *R. nigricans* and some of the other species as to their capacity to infect sweet potatoes.

*R. tritici* was selected, first, because it is the species most commonly isolated from sweet potatoes next to *R. nigricans*, and, second, because with the exception of *R. nigricans* it is the species most commonly received in response to requests to other investigators for cultures of *Rhizopus*. It has been sent in, on a number of occasions, as *R. nigricans*.

*R. oryzae* was selected because Hanzawa (2) placed it in the high-temperature group (*R. tritici* falling into the intermediate and *R. nigricans* into the low-temperature group) with the idea of studying one organism of each thermal group.

*R. reflexus* was chosen because it belongs to the same thermal group as *R. nigricans*, its upper temperature limit being slightly higher than that of *R. nigricans* and its lower temperature limit slightly lower. Furthermore, it decays sweet potatoes (where the "well" method is used) fully as rapidly as *R. nigricans*.

*R. artocarp*i was likewise chosen because it belongs to the same thermal group as *R. nigricans* and is able to decay sweet potatoes over much the same temperature range, where the "well" method of inoculation is used.

The spore suspensions employed in these experiments were made by introducing spores from cultures of the respective species into battery jars containing water. In every case except that of *R. nigricans* the concentration was so great as to render the water almost black.

The reason for using such highly concentrated spore suspensions was to insure ample competition for *R. nigricans*, which, in the first and second series of experiments, successfully excluded *R. tritici* from infecting potatoes at temperatures below 20° C. and which in the course of these experiments proved to be able to compete against odds with the other species.

<sup>1</sup> It is not practical to directly compare the amount of decay caused by one species with that of another because of the indeterminable factors involved, but the indications are that the rate of decay by *R. tritici* is as rapid as that of *R. nigricans* at most temperatures below 20° C.

The spore suspensions of *R. nigricans* used in connection with some of the other species was not so highly concentrated, because it so happened that there was not a sufficient number of cultures of *R. nigricans* available.

Table IV shows the results of competitive tests of infection by *R. tritici* and *R. nigricans*, first when potatoes were washed and inoculated with *R. tritici*, second, when unwashed potatoes were inoculated with *R. tritici*, and third, when unwashed potatoes were inoculated with a spore suspension of *R. tritici* and *R. nigricans*. Controls of both washed and unwashed potatoes were also included.

*R. nigricans* alone was isolated from the unwashed and control potatoes and greatly predominated when either the washed potatoes were inoculated with *R. tritici* or unwashed potatoes were inoculated with a mixed spore suspension of *R. tritici* and *R. nigricans*. These results indicate that *R. tritici* can not compete with *R. nigricans* and is not much of a factor in the decay of sweet potatoes at these temperatures. The number of spores of *R. nigricans* on the potatoes as they were obtained from the storage house or when the potatoes were washed, was sufficient to cause nearly all the infection, even when the potatoes were inoculated with a highly concentrated spore suspension of *R. tritici*. Washing reduces infection by *R. nigricans* but slightly.

Table V shows that *R. artocarp*i can compete more successfully with *R. nigricans* than *R. tritici*. When *R. artocarp*i is used alone as the inoculum, the percentage of infection is fairly high, especially at 14° C. The washing of the potatoes seems to have no effect. When the inoculations were made with *R. nigricans* and *R. artocarp*i together, no infection by the latter organism took place. *R. nigricans* alone was isolated from the controls.

Although these results show that *R. artocarp*i may cause considerable decay if spore suspensions of high concentration are used, it seems probable that under storage and transit conditions the number of spores of *R. artocarp*i would rarely if ever be sufficient to be a factor in the decay of sweet potatoes, because *R. nigricans* seems to be universally present and would likely grow and produce spores as readily as *R. artocarp*i under most, if not all, conditions.

*R. reflexus*, as is shown in Table VI, was capable of causing considerable decay when it was used alone as the inoculum.

TABLE IV.—Organisms isolated from sweet potatoes inoculated with *Rhizopus tritici* or with a mixed spore suspension of *Rhizopus tritici* and *Rhizopus nigricans*, or not inoculated and held as controls

Temperature.	Inoculated with <i>Rhizopus tritici</i> .					Inoculated with <i>Rhizopus tritici</i> and <i>Rhizopus nigricans</i> (unwashed).		Control.	
	Washed.			Unwashed.		<i>Rhizopus tritici</i> .	<i>Rhizopus nigricans</i> .	Washed.	Unwashed.
	<i>Rhizopus tritici</i> .	<i>Rhizopus nigricans</i> .	<i>Rhizopus tritici</i> and <i>Rhizopus nigricans</i> mixed.	<i>Rhizopus tritici</i> .	<i>Rhizopus nigricans</i> .			<i>Rhizopus nigricans</i> .	<i>Rhizopus nigricans</i> .
°C.									
.....	2	13	2	0	13	2	6	14	17
.....	1	25	0	0	13	0	3	12	16

TABLE V.—Organisms isolated from sweet potatoes inoculated with *Rhizopus artocarp* or with a mixed spore suspension of *Rhizopus artocarp* and *Rhizopus nigricans*, or not inoculated and held as controls

Temperature.	Inoculated with <i>Rhizopus artocarp</i> .						Inoculated with <i>Rhizopus artocarp</i> and <i>Rhizopus nigricans</i> (potatoes washed).		Control.	
	Washed before inoculation.			Unwashed.			Washed.	Unwashed.	Washed.	Unwashed.
	<i>Rhizopus artocarp</i> .	<i>Rhizopus nigricans</i> .	<i>Rhizopus artocarp</i> and <i>Rhizopus nigricans</i> (mixed).	<i>Rhizopus artocarp</i> .	<i>Rhizopus nigricans</i> .	<i>Rhizopus artocarp</i> and <i>Rhizopus nigricans</i> mixed.				
°C.										
14. . . . .	21	11	3	16	9	2	43	2 R t and R n and 2 R.t. . . . .	16	16
12 . . . . .	9	16	1	7	26	.	16	...	15	17

TABLE VI.—Organisms isolated from sweet potatoes inoculated with *Rhizopus reflexus* or with a mixed spore suspension of *Rhizopus reflexus* and *Rhizopus nigricans*, or not inoculated and held as controls.

Temperature.	Inoculated with <i>Rhizopus reflexus</i> .						Inoculated with <i>Rhizopus reflexus</i> and <i>Rhizopus nigricans</i>		Control.	
	Washed before inoculation			Unwashed			Washed.	Unwashed.	Washed.	Unwashed.
	<i>Rhizopus reflexus</i> .	<i>Rhizopus nigricans</i> .	<i>Rhizopus reflexus</i> and <i>Rhizopus nigricans</i> (mixed).	<i>Rhizopus reflexus</i> .	<i>Rhizopus nigricans</i> .	<i>Rhizopus reflexus</i> and <i>Rhizopus nigricans</i> mixed.				
°C.										
14. . . . .	12	17	0	10	16	2	22	15	14	17
12. . . . .	17	14	2	16	15	1	8	15	12	30

This species caused no infection, however, when *R. nigricans* was included in the inoculum. Washing the potatoes did not influence the results. These results indicate that *R. reflexus* probably is not normally a factor in the decay of potatoes at storage temperatures.

The results recorded in Table VII show that *R. oryzae*<sup>a</sup> falls into the same group as *R. tritici* with regard to the amount of infection at these temperatures. The only infection that took place was when the potatoes were washed. This fact may have had no relation to washing and may have been accidental, for in the cases of *R. artocarp* and *R. reflexus*, washing had no effect upon the amount of infection. The effects of

<sup>a</sup> It is assumed for two reasons that *R. oryzae* was the organism that caused the infection, first, because such a highly concentrated spore suspension was used in the inoculation of the potatoes, second, because *R. tritici* or the other species belonging to this group never have been known to cause infection when it was not used in the inoculum, except in the one instance shown in Table V in the case of *R. tritici*.

washing in connection with these experiments and those with *R. tritici* are so small as to fall within the limits of experimental error.

The results recorded in Tables IV, V, VI, and VII show: First, that *R. tritici* and *R. oryzae* can not compete with *R. nigricans* at temperatures of 12° and 14° C. in the infection of sweet potatoes, even where high concentrations of spores of these organisms are used in the absence of *R. nigricans* from the inoculum; second, although *R. artocarp*i and *R. reflexus* are more successful than *R. tritici* and *R. oryzae* in competition with *R. nigricans*, they can not compete when the latter is included in the inoculum; third, *R. tritici*, *R. oryzae*, *R. artocarp*i, and *R. reflexus* do not infect sweet potatoes at these temperatures when infection depends upon the organisms present on the potatoes, while *R. nigricans* does.

TABLE VII.—Organisms isolated from sweet potatoes inoculated with *Rhizopus oryzae*, or not inoculated and held as controls

Temperature.	Number of cultures isolated.			Remarks.
	Potatoes inoculated with <i>Rhizopus oryzae</i> .			
	Washed before inoculation.		Unwashed.	
	<i>Rhizopus nigricans</i> .	<i>Rhizopus oryzae</i> and <i>Rhizopus nigricans</i> mixed.	<i>Rhizopus nigricans</i> .	
° C.				
14 . . . . .	14	2	13	Same control as in Table V.
12 . . . . .	13	. . .	14	

When comparing the results from experiments with *R. tritici* and *R. oryzae* with those obtained with *R. artocarp*i and *R. reflexus* some reservations must be made because it is not known that the concentration of the spore suspensions are equal or comparable. The differences in the amount of infections are so great that they would seem to be due to differences in capacity to infect, rather than to differences in concentration. The concentration was so great in every case that it would seem that a small variation in concentration would alter but little the amount of infection by the particular species used in the inoculum. In fact, very little infection occurred under any circumstances with *R. tritici* and *R. oryzae*. It is reasonable to expect that *R. reflexus* and *R. artocarp*i, especially the former, would cause more infection at these temperatures than *R. tritici* and *R. oryzae*, since the former are low, while the latter are high temperature forms.

It will be seen from Tables V and VI that it may be important in studies of resistance and susceptibility to take into consideration the temperatures at which comparisons are made. For instance, *R. artocarp*i is more successful in competition with *R. nigricans* at 14° than at 12° C., while *R. reflexus* is more successful at 12° than at 14°. These results are consistent with other temperature relations of these organisms, which show that the lower temperature limit for infection with *R. reflexus* is lower than for *R. artocarp*i.

In only one instance has *R. tritici* (Table V) been isolated from sweet potatoes at storage temperatures when the potatoes became infected, in the absence of this organism in the inoculum. It is to be expected that *R. tritici* would be responsible for a small percentage of infections,

since it can infect at these temperatures and probably is nearly always present on the potatoes. (Table I.)

The fact that *R. nigricans* was always obtained from the controls as well as inoculated potatoes in previous experiments when the temperature conditions were right indicates that this species is usually, if not always, present on the potatoes.

#### TYPE FOUR

In the fourth type of experiments the potatoes were wounded as in the preceding experiments, but inoculated differently. The first inoculation was made with a suspension of spores of *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarp*i, taken from two cultures of each species of the same age and grown under identical conditions, suspended in 3 liters of water; the second inoculation was with a suspension of spores of *R. tritici*, *R. oryzae*, *R. reflexus*, *R. artocarp*i, and *R. nigricans*, two cultures of each of the first four species and one of *R. nigricans*. The same amount of water as in the first case was used in making the inoculum.

The object of these experiments was to determine which species would infect sweet potatoes when several species were in competition with each other at storage temperatures. The number of spores of each species present in the inoculum only approximated that of the other species, and in the case of *R. nigricans* was probably much less. Of course, as has been shown in previous experiments, *R. nigricans* is usually, if not always, present, but it is believed that, at least when the potatoes were not inoculated with *R. nigricans*, the number of spores of this species present per unit area was less than that of the other species, because the concentration of the spores in the inoculum of the other species was fairly high.

It did not seem practical at the time to attempt to use exactly equivalent concentrations because of the difficulties involved. The most serious difficulties were, first, to obtain an equal number of spores of each species, second, the number of spores may not be a measure of the germinating and infecting capacity of the spores, which in turn may vary with the species, and, latterly, some variation in the germinating and infecting capacity of the spores of the different species may be expected under different conditions, as, for example, at different temperatures, and yet a comparison between species must be made under identical conditions to be valid. This is shown to be the case by the fact that, although the temperature ranges of *R. reflexus* and *R. artocarp*i run almost parallel, *R. reflexus* is the more successful in competition with *R. nigricans* at 12° C., while at 14° *R. artocarp*i is the more successful (Tables V and VI). It is true that the temperature range of *R. reflexus* is wider than that of *R. artocarp*i, but its maximum is about the same number of degrees above that of *R. artocarp*i as its minimum is below. This difference in temperature ranges is, perhaps, sufficient to account for their difference in infective power. It probably would be difficult to find two fungi of identical temperature ranges. To make a quantitative comparison these factors must be taken into consideration.

It is believed, however, that the results from the methods employed in these experiments will show which species of *Rhizopus* are important in the decay of sweet potatoes.

Table VIII shows the number of isolations obtained from potatoes inoculated with *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarp*i, and from potatoes inoculated with the same organisms plus *R. nigricans*, at the temperatures 14° and 18° C. It will be noted that at 18° *R. artocarp*i



was isolated from 17 potatoes as compared with *R. nigricans* from 4, when *R. nigricans* was not included in the inoculum. On the other hand, *R. artocarp*i was obtained from only 6 potatoes as compared with *R. nigricans* from 11 (and in 3 of these cases the isolations were a mixture of *R. artocarp*i and *R. nigricans*) when *R. nigricans* formed a part of the inoculum. At 14° quite a different relationship was found. When *R. nigricans* was excluded from the inoculum, *R. artocarp*i was isolated from 6 potatoes (4 of these isolations were a mixture of *R. artocarp*i and *R. nigricans*), *R. nigricans* from 29 and *R. reflexus* from 3. When *R. nigricans* was included in the inoculum, *R. artocarp*i was isolated once, a mixture of *R. artocarp*i and *R. nigricans* twice, and *R. nigricans* 33 times. These results show, first, that the percentage of infection by *R. artocarp*i was higher when *R. nigricans* was excluded from the inoculum, although at 14° the difference was small; second, that 18° was more favorable for infection by *R. artocarp*i than 14°. This was especially evident when *R. nigricans* was excluded, in which case the infection by *R. artocarp*i greatly exceeded that of *R. nigricans*; third, that a temperature of 14° was more favorable for *R. reflexus* than one of 18°; fourth, that *R. artocarp*i showed a slightly higher percentage of infection at 14° than *R. reflexus* (these results correspond to those recorded in Tables V and VI); fifth, that *R. tritici* and *R. oryzae* at these temperatures infect less readily than either *R. artocarp*i or *R. reflexus*; and sixth, that *R. nigricans* infects more readily than any of the other species. This is shown, even when infection by *R. nigricans* is compared with that of *R. artocarp*i at 18°, because although *R. artocarp*i caused the greater part of the decay when *R. nigricans* was excluded from the inoculum, the reverse was true when *R. nigricans* was included. This was true notwithstanding the fact that two cultures of *R. artocarp*i were used as compared with one of *R. nigricans*. Even assuming that spores of *R. nigricans* on the potatoes were equal in number to the *R. artocarp*i spores in one culture and the number of spores in the cultures of the two were equal, the margin is much in favor of *R. nigricans*. It is believed that there is a greater number of spores in a culture of *R. artocarp*i than in a culture of *R. nigricans*.

The percentage of infection by *R. artocarp*i and *R. reflexus* as compared with *R. nigricans* is not as high in these experiments as is shown in Tables V and VI at 14° C., but the concentration of the spores in the inoculum was not as high in the latter as in the former case.

TABLE VIII.—Organisms isolated from wounded sweet potatoes inoculated with a mixed suspension of spores of a number of species of *Rhizopus*

Organisms employed.	Temperature.	Number of isolations.	Organisms isolated.
<i>Rhizopus tritici</i> , <i>oryzae</i> , <i>reflexus</i> and <i>artocarp</i> i.....	°C. 18	21	17 <i>R. artocarp</i> i. 4 <i>R. nigricans</i> .
	14	38	2 <i>R. artocarp</i> i. 4 <i>R. artocarp</i> i and <i>nigricans</i> , mixed. 29 <i>R. nigricans</i> . 3 <i>R. reflexus</i> .
	18	17	3 <i>R. artocarp</i> i. 3 <i>R. artocarp</i> i and <i>nigricans</i> , mixed. 11 <i>R. nigricans</i> .
	14	36	1 <i>R. artocarp</i> i. 2 <i>R. artocarp</i> i and <i>nigricans</i> , mixed. 33 <i>R. nigricans</i> .

## DISCUSSION OF RESULTS

*R. tritici* and *R. nigricans* are the two species chiefly responsible for the decay of sweet potatoes, known as softrot. The former is responsible for decay at the higher and the latter at the lower temperature, while the two overlap between 20° and 30° C. Although other species are capable of causing softrot, they do not seem to do so under the storage conditions at Washington, D. C., and in the infection chambers at the different temperatures. *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarpi* cannot compete successfully with *R. nigricans* at temperatures of 12° and 14° when sweet potatoes are inoculated with any one of these organisms along with *R. nigricans*. Even though high concentrations of spores of *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarpi* are used alone, *R. nigricans* nearly always causes more decay than any of them, and *R. tritici* and *R. oryzae* cause very little under such conditions.

When a mixed spore suspension of *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarpi* is used in inoculating the potatoes held at temperatures of 14° and 18° C., *R. artocarpi*, *R. reflexus*, and *R. nigricans*, with *R. nigricans* greatly predominating, were isolated at 14°, and *R. artocarpi* and *R. nigricans* at 18°, *R. artocarpi* greatly predominating. When *R. nigricans* was added to the inoculum just mentioned, *R. nigricans* and *R. artocarpi* were the only species isolated, with the former greatly predominating.

These results show that *R. nigricans*, except in the case of *R. artocarpi* at 18° C. (when *R. nigricans* was not included in the inoculum), was the chief agent of decay, even in the presence of the highly concentrated spore suspension of the other species used in these experiments.

Why *R. nigricans* should cause so much decay when such high concentrations of spores of the other species were employed, especially in the cases of *R. reflexus* and *R. artocarpi*, cannot be explained now. This seems rather strange when it is considered that *R. reflexus*, in particular, seems to decay sweet potatoes fully as rapidly as *R. nigricans* when the "well" method of inoculating is employed at the same temperatures. The two cases are hardly comparable, however, since in the present experiments we are dealing with the percentage of infection, while in the case cited we have to do with the rapidity of decay after infection has occurred. When the "well" method is used there is nearly always 100 per cent infection, irrespective of the species employed. This method is not at all comparable, however, to the one employed in these experiments.

When these results are compared with those obtained by Harter, Weimer, and Lauritzen (6), who showed that several species of *Rhizopus* have the capacity to decay sweet potatoes, one should take into consideration the methods employed in the two cases. In the former case the particular species involved in infection was not only present as a concentrated mass of active mycelium, but had the additional advantage of having access to the available food still remaining in the decoction. The species concerned also had access to the food rendered available soon after inoculation by the action of the pectinase, present in the decoction, upon the middle lamellae (3). In the latter case, infection depended either upon the spores present on the potatoes as they were removed from storage, or upon the spores introduced as a suspension in water. Where the potatoes were not inoculated, the species present may have been limited to those (*R. tritici* and *R. nigricans*) isolated in the first type of experiments. If other species were present, they were

unsuccessful in competition with these two species. When the potatoes were inoculated as above, the competition between the species present, whether introduced or occurring normally, was more nearly equal than it was between the species present on the potatoes as they came from storage and the inoculum used in connection with the "well" method.

It should be realized from a comparison of the results obtained by the two methods, that because a fungus produces a disease under one set of experimental conditions, it does not necessarily do so under another. The factors involved in determining infection under experimental conditions may vary greatly from those under normal conditions, depending upon, first, whether or not the experiments are designed to approximate normal conditions, and, second, whether or not the circumstances and our knowledge enable us to recognize and control the factors involved.

There seems to be some unknown factor or factors, either as a part of the capacity to decay, or associated with it, on the part of *R. nigricans* that enables it under normal conditions or the conditions of these experiments to infect sweet potatoes to the exclusion of the other species.

#### SUMMARY

(1) *Rhizopus nigricans* and *R. tritici* are the species primarily responsible for the decay of sweet potatoes known as softrot, *R. nigricans* at temperatures between 6° and 20° C., and *R. tritici* at 30° and above, the two overlapping between 20° and 30°.

(2) The temperature range of infection by *R. tritici*, *R. nigricans*, and *Mucor* is so wide as to exclude the possibility of storing sweet potatoes beyond the limits of this range.

(3) *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarpi* can not compete successfully with *R. nigricans* at the temperatures of 12°, 14°, and 18° C.

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# THE INHERITANCE OF GROWTH HABIT AND RESISTANCE TO STEM RUST IN A CROSS BETWEEN TWO VARIETIES OF COMMON WHEAT <sup>1</sup>

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## INTRODUCTION

There are three general methods by which the tremendous destruction of wheat by black stem rust may be reduced. These are the eradication of the common barberry (13),<sup>3</sup> the growing of rust-resistant varieties (1, 10), and the use of improved methods of field culture (14). The losses have been especially severe in the hard red spring wheat area. No hard red spring wheat of high quality and yield, which is generally resistant to stem rust in the hard spring wheat belt, is now grown on a large acreage. Kota, apparently, is highly resistant to stem rust in those districts in which it has been grown, but on account of weak straw it is likely to lodge in the more humid sections. Therefore, the importance of developing other rust-resistant hard red spring wheats is quite apparent.

The production of varieties of wheat resistant to stem rust is a complex problem. Until recently it was supposed that only one form of stem rust caused the epidemics on wheat, although some of the early workers believed that the parasitic capabilities of the rust were easily modified (2). However, Stakman and Piemeisel (17), followed by others (8, 9, 15) have shown that *Puccinia graminis tritici* Erikss. and Henn. in reality consists of many biologic forms which differ in their pathogenicity for certain varieties of wheat.

This discovery explains why the same variety of wheat may be resistant when grown in one locality and susceptible when grown in another, or why a variety may be resistant in the same locality in one year and susceptible in the next. It is obvious that if a wheat is to be resistant in the field it must be resistant to all of the biologic forms present in the locality in which it is to be grown.

Both Kanred and Marquis are good milling and high yielding wheats. Kanred, however, is practically immune from several biologic forms of stem rust to which Marquis is susceptible. The purpose of this paper is to present data regarding the mode of inheritance of growth habit (winter versus spring habit) and resistance to stem rust in a cross between Kanred and Marquis wheats.

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<sup>2</sup> This work on the development of rust-resistant varieties of wheat was under the direction of Dr. H. K. Hayes and Dr. E. C. Stakman. The cross was made in 1918 by Carl Kurtzweil and others. The seed of the first generation was furnished by Doctor Hayes. The biologic forms of rust were supplied by Doctor Stakman and Mr. M. N. Levine.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," pp. 468-469.

Hayes, Parker, and Kurtzweil (7) recently studied the inheritance of resistance and susceptibility, to what apparently was a single biologic form, in crosses between common and durum wheats and common wheat and emmer. They showed that the inheritance of resistance and susceptibility to stem rust was not the same in different crosses. In the durum-common cross, susceptibility appeared to be dominant while in the emmer-common cross the  $F_1$  was resistant, but not as resistant as the emmer parent. There was some linkage in transmission between the emmer and durum types and resistance to stem rust. Resistant emmer and durum types were very common, while it was quite difficult to obtain resistant common wheats.

Puttick (11) reported the results of a study on the reaction of the  $F_2$  plants of a cross between two varieties of wheat which react reciprocally to two biologic forms of stem rust. Many gradations in reaction to both forms of rust appeared, varying from complete susceptibility to complete immunity.

Extensive rust surveys have been made during the last few years. The prevalence, distribution, and virulence of the various biologic forms of *Puccinia graminis tritici* are now being ascertained and, for all practical purposes, the parasitic effect on the hosts seems to be constant (16). The plant breeder now has a definite basic foundation for the development of varieties resistant to stem rust.

Twenty-one biologic forms<sup>4</sup> of rust have been found in the upper half of the Mississippi Valley. As the winter wheat, Kanred, is resistant to 2 of these forms and immune from 11, the value of this variety in breeding a rust-resistant spring wheat is apparent. If there could be isolated from the progeny of a Kanred-Marquis cross, a spring wheat which would be resistant to some or all of the forms to which the winter parent is resistant, one step would be accomplished of the many needed to produce a generally resistant variety.

Kanred is a true winter wheat which, when sown in the spring, at University Farm, St. Paul, Minn., produces only an occasional head late in the season and does not set seed. Marquis is a true spring wheat, which, when sown in the fall, seldom, if ever, lives through the winter. Growth habit, as used in this paper, is meant to indicate that general difference which exists between true spring and true winter varieties in their ability to produce heads normally when sown in the spring of the year (Pl. 1, A).

Apparently few investigations have been made on the inheritance of growth habit. Innumerable observations have been reported in literature on the differences in heading period and maturity between varieties in both spring and winter groups. These differences, while they may be of the same general nature as winter habit, are comparatively very minute, but they are constant, as was shown by Fruwirth (3) in 1918.

A single head of wheat was selected and divided into two parts. One-half of this seed and its progeny was constantly sown in the fall and the other half in the spring. This process was continued for 8 years. The two lots of seed were then sown together in both the fall and spring and the growth habits compared. The period of blossoming and ripening was the same for all plots, showing that selection within a pure line was of no value.

<sup>4</sup>Unpublished data furnished by E. C. Stakman and M. N. Levine.

The nature of the processes involved in bringing about the heading period has been a matter of speculation for some time. The climatic units which control these processes and permit winter grains to produce seed are finely interwoven. Although temperature and moisture are very important factors in the growth and maturing of plants, it is quite evident from the plant life about us that the time of flowering and fruiting of most plants is definitely connected in some way with the advance of the season. Garner and Allard (6) have clearly demonstrated this third factor to be the change in length of day and night. They found that certain plants which ordinarily require a short day for flowering and fruiting could be induced to flower and fruit in the middle of the summer by shortening the length of day to that which was normal for the regular flowering season. This was done by placing the plants in a dark-house for a certain number of hours each day.

In contrast to this group of plants which require a short day for flowering and fruiting, is that group of plants which require a long day of light. These plants flower regularly in the late spring or early summer. Garner and Allard place our small grains in this group.

As regards the inheritance of the growth-habit character, the results reported by previous workers do not appear at first to be in full agreement. Spillman (12) reported in 1909, that the winter character was dominant over the spring character in a cross between a winter common wheat and a spring club wheat. Fruwirth (4, p. 176) in 1910, cites Tschermak as having reported that the winter type was dominant over the spring type. When sown in the fall, the first generation of hybrids wintered over somewhat better than the true winter forms; but when sown in the spring, they remained dormant through nearly the whole summer. Single shoots appeared and began to blossom, but they produced no seed.

Gaines (5, p. 42-45) in 1917, reported that he obtained a segregation of spring and winter types from a cross between 2 spring varieties of barley, Rice and Beardless. The  $F_1$  when sown in the spring, headed normally. In the  $F_2$ , there was a ratio of 3 winter plants to 13 spring plants. The plants in the third generation bred fairly close to expected ratios. He found that seasonal variations influenced the heading periods and consequently the ratios. The segregation in the  $F_2$ , however, indicates a dominance of the spring type over the winter type.

A complete and detailed study of the genetic nature of growth habit in wheat varieties has been made recently by Vavilov and Kouznetsov (18). They crossed a common winter wheat with a club spring wheat and found a clear dominance of the spring character over the winter character. There was a complicated segregation in the  $F_2$ , and some of the segregates (including many intermediates) were homozygous in the  $F_3$ . Of the 552  $F_2$  plants, 500 were early or late spring plants and 52 were typical winter plants. The results obtained by the writer on the inheritance of spring and winter habit are quite in accord with those of Vavilov and Kouznetsov.

#### MATERIALS AND EXPERIMENTAL METHODS

Marquis, a hard red spring common wheat of high quality, was crossed with Kanred in the summer of 1918. The latter, which is immune from several different biologic forms of stem rust, is a high yielding selection from Crimean hard red winter wheat.

The crossed seed was sown in the fall of the same year and produced 80 plants, 2 of which were winterkilled and 5 of which were not crosses. The remaining plants were harvested individually and the seed sown in the spring of 1920.

In the second generation a population of approximately 5,000 plants was grown. In order to facilitate observations the seeds were sown at intervals of 3 inches, in rows 1 foot apart. The date of emergence of the first head on each plant was noted. The hybrids formed a continuous series for date of head emergence, beginning with those which came out at the same time as the spring parent, to those which did not emerge at all. In this way they resembled the winter parent. (Pl. 1, B.)

The time of heading was divided into weekly periods. One week from the day on which the first plant headed, tags were placed on plants on which one or more heads had emerged. These comprised the first class. All of the Marquis control plants headed during the same period as did those plants included in the first class. One week later, tags were attached to all plants which had headed since those of class 1. These constituted the second class. This process was continued for 8 weeks, after which period no more plants headed. The plants which did not head were classed as true winter types. The winter parent controls failed to head, thereby falling into the same class as the winter hybrids.

From the first 7 heading classes in the  $F_2$ , 65 families were grown in the  $F_3$ . The plants from the seventh class produced only a few seeds, while those of the eighth class headed so late that no seed was produced at all. In sowing, the seed was again spaced as in the second generation so that a study could be made of the individual plants.

Several of the families were uniform for heading period in the  $F_3$ . In these cases the entire plot was given a general heading date, as in a varietal test. Others of the families were heterozygous for date of head emergence. In those plots a final count was made at harvest time of the number of plants which failed to head and the number which produced heads.

The rust studies were made on plants growing in the field under an artificial epidemic produced with several different biologic forms of stem rust and also on inoculated seedlings in the greenhouse.

In the second generation all the plants were grown in the field under an artificial epidemic produced by spraying the plants with a suspension of urediniospores of several different biologic forms. Both parents were susceptible to some of the rust forms which were used. All of the hybrid material was as susceptible as either parent in this epidemic and therefore a detailed genetic study of the inheritance of rust resistance under field conditions could not be made. Studies on the inheritance of resistance and susceptibility were made in the greenhouse by inoculating the  $F_3$  seedlings with cultures of urediniospores of known biologic forms.

The seedlings in the greenhouse were grown in 4-inch pots and inoculated when they were  $1\frac{1}{2}$  to 2 inches tall. After inoculation they were placed in a glass-topped chamber and incubated 48 hours. The notes on infection were taken 12 to 14 days after the date of inoculation. Some of the plants were completely susceptible and the others were immune since there were no intermediate types of infection. The uredinia were large, coalescing, and normal in every respect on the seedlings both of Marquis and of susceptible hybrids, while the seedlings of Kanred and

of the resistant hybrids were immune. There were, therefore, only two classes, i. e., immune and susceptible.

An intensive study was made by testing a large number of  $F_2$  plants with a single known biologic form to which the Marquis parent is susceptible and the Kanred parent is resistant. Two questions then naturally arise: How is the reaction tendency of the host to all of these biologic forms inherited? Is the reaction due to the presence of a single genetic factor, or, if several factors are concerned, are they linked in the process of segregation? An attempt was made to solve these questions by inoculating various  $F_2$  selections, which were homozygous in their reaction to the first form studied, with 12 other biologic forms of stem rust.

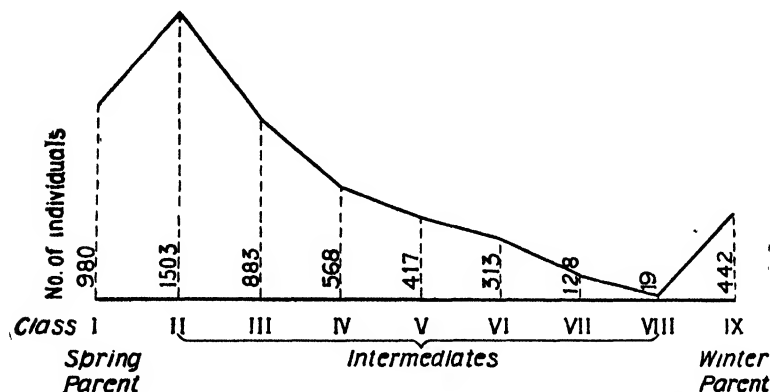


Fig. 1.—Diagram showing the segregation for time of heading in the spring-sown  $F_2$  of a Marquis  $\times$  Kanred hybrid.

## EXPERIMENTAL RESULTS

### THE MODE OF INHERITANCE OF GROWTH HABIT

The first generation material was sown in the fall. Because of the mildness of the season, only 2 out of 75 plants were killed during the winter. Had the winter been severe, it is possible that a higher percentage of the first generation plants would have been killed. No  $F_1$  plants were grown from spring-sown seed.

All of the seed from the  $F_1$  was sown in the spring of the following year, producing an  $F_2$  population of 5,250 plants. Of this number 4,808 plants headed during the summer and 442 plants did not. Of the former, 980 plants headed at the same time as did the spring parent. In the second weekly period 1,502 plants headed, which is the greatest number to fall into any one heading period. From the second period on to the eighth, there was a gradual decrease in the number of plants heading. In the eighth period there were but 19 plants. The total number for each class is shown in figure 1.

It is very evident from the chart that the segregation of the plants for growth habit characters in the  $F_2$  is of a complex nature. All of the plants in the first 5 classes matured like any ordinary spring wheat variety. It would be quite fair, then, to assume that in general, the plants in these



first 5 classes could represent reasonably well the true spring type. If such were the case, there would be 4,350 plants of the spring type and 900 of the winter, a ratio of approximately 5 spring plants to 1 winter plant. If it were assumed that all plants which headed during the summer were of spring type, there would be a ratio of approximately 11 spring to 1 winter. In either case there is a partial dominance of the spring over the winter habit.

A study was made to ascertain whether any correlation existed between the inheritance of growth habit and the presence of awns. A total of 432 plants were tabulated according to their growth habit in relation to this character. The awn characters were recorded from the  $F_2$  individual plants and controlled by the progeny performance in the  $F_3$ . The results are presented in Table I.

TABLE I.—*The relation between awns and growth habit in the  $F_2$  progeny of a Kanred  $\times$  Marquis hybrid*

Heading period.	Number of plants with long awns.	Number of plants with intermediate awns.	Number of awnless plants.
1.....	18	25	14
2.....	17	29	11
3.....	14	21	16
4.....	21	21	24
5.....	23	26	16
6.....	29	41	38
7.....	6	9	11
8.....	1	.....	1
Total.....	129	172	131

The results presented in Table I show that there is a lack of correlation in the inheritance of awns and growth habit characters. While the numbers are not very great for each separate heading period, there is a total of 129 bearded plants compared to 131 beardless plants for the first 8 periods.

The segregation of plants for growth habit in the  $F_3$  families was in accord with the segregation obtained in the  $F_2$ . The growth habit of the plants belonging to the various  $F_2$  groups is shown in Table II.

TABLE II.—*The growth habit in  $F_3$  of plants belonging to separate  $F_2$  heading groups in a Marquis-Kanred hybrid*

Heading period.	Number of families.			
	Grown.	Homozygous for spring habit.	Heterozygous for growth habit.	Homozygous for winter habit.
1.....	10	10	0	0
2.....	10	6	4	0
3.....	10	7	3	0
4.....	10	5	5	0
5.....	10	2	8	0
6.....	9	0	9	0
7.....	6	0	4	2

The plants selected from the first heading period in the second generation were homozygous for the spring habit of growth in the third generation. Beginning with the plants in the second heading period, there was a gradual decrease in the number of families which were homozygous for the spring character and a corresponding increase in the number of families heterozygous for the same character as the seventh heading period was approached. There was also a gradual change in the ratios of spring to winter types progressing from the first to the seventh heading period. In the seventh period, 2 families were homozygous for winter character. If the plants in the eighth class had produced seed in the second generation, there undoubtedly would have been a still larger number of homozygous winter types in proportion to the number of spring types.

One can readily see from the data presented that there is a great difference in degree of heterozygosity of the plants in the various heading periods. In the first heading period all 10 of the  $F_2$  families are homozygous for spring type; in the second, 6 of 10; in the third, 7 of 10; in the fourth, 5 of 10; in the fifth, 2 of 10; in the sixth, all 9 of the families are heterozygous for growth habit, and in the seventh period 4 families are heterozygous for growth habit and 2 are homozygous for winter type. With the exception of those from the sixth heading period, homozygous forms were obtained in all classes grown. A number of  $F_3$  families were homozygous spring types comparable to our ordinary hard red spring wheat varieties. In addition to these early-heading plants, a few families were obtained which were homozygous for a heading period much later than that of the Marquis parent.

From Table III it will be noted that there is a correlation between the  $F_2$  heading period and the percentage of spring types produced by the various  $F_3$  families.

TABLE III.—Showing the growth habit of the  $F_3$  from  $F_2$  plants heterozygous for growth habit

Heading period	Number of types		Spring types.
	Spring.	Winter.	
I.....	All.	None.	Per cent. 100
2.....	154	13	92.2
3.....	121	14	89.6
4.....	158	39	80.2
5.....	236	46	83.7
6.....	187	69	73
7.....	12	6	66.7

With the exception of the very slight increase in the fifth heading period, there is a very regular decrease in the percentage of spring types to winter types as one proceeds from the first to the seventh heading period. Here also there undoubtedly would have been a more complete reversal of the ratios of spring types to winter types in plants of the eighth and ninth heading periods, had it been possible to grow these plants in the third generation.

## THE MODE OF INHERITANCE OF RUST RESISTANCE

The  $F_1$  plants grown in the field during the summer of 1919 were not infected, since no rust developed in the field where they were grown. Consequently no determinations of resistance or susceptibility could be made under field conditions.

The  $F_2$  plants were extremely susceptible when grown in the field under an epidemic produced artificially by inoculation with several biologic forms. The Marquis control plants, which are completely susceptible to all of the forms used in producing the epidemic, had an average rust infection of 87.7 per cent. The hybrids growing under the same conditions had an average rust infection of 80 per cent. This high susceptibility of the hybrids growing in the field was to be expected, because both the Marquis parent and the Kanred parent are susceptible to some of the forms used in producing this epidemic. These results very clearly demonstrate how a general field epidemic may fail to differentiate the segregation for resistance and susceptibility in the progeny from a given cross. The most accurate and reliable method of determining the resistance and susceptibility of hybrid progeny to any given number of biologic forms is to grow the plants under controlled conditions and to inoculate them with single known biologic forms.

The plants of 10 families from each of the first six classes, and 5 families from the seventh class, for growth habit (fig. 1), were tested for their reaction to Biologic Form I. This form is one which has been carried in pure culture since 1916 and has remained constant in its reaction on various host plants throughout this period. Of the 65 families tested, 23 were pure for resistance to this form of rust, 10 were susceptible, and 32 were heterozygous. The ratios are not very significant when taken from such a small number of families. There is a numerical ratio of 23 resistant families, 32 heterozygous, and 10 susceptible. The ratio of homozygous to heterozygous families is a very close approximation to the expected 1:1 ratio. The inoculation results are given in Table IV.

TABLE IV.—The reaction of Marquis and Kanred, and various families of the  $F_2$  from a cross between Kanred and Marquis, to Biologic Form I of stem rust<sup>1</sup>

Class.	Homozygous for resistance.		Heterozygous for resistance.			Homozygous for susceptibility.	
	Number of families.	Number of individuals.	Number of families.	Number of individuals resistant.	Number of individuals susceptible.	Number of families.	Number of individuals
1.....	3	102	4	96	26	3	82
2.....	3	75	7	184	54	0	0
3.....	3	108	4	102	35	3	88
4.....	1	21	8	239	59	1	26
5.....	4	111	4	89	28	2	73
6.....	6	140	3	65	18	1	24
7.....	3	50	2	23	2	0	0
Total.....	23	607	32	798	222	10	293
Marquis.....		0		0	0		83
Kanred.....		83		0	0		0

<sup>1</sup> A total of 2,086 individual plants were inoculated. The resistant hybrid plants were just as free from lesions as the resistant Kanred parent. The susceptible hybrid plants were completely susceptible, producing large, vigorous, and confluent uredinia. This type of infection was like that obtained on the Marquis parent (Pl. 2).

There were 1,020 individuals in the 32 families which were heterozygous in their reaction to Biologic Form I. Of this number, 798 were immune, while 222 plants were clearly susceptible, an approximate ratio of 3 resistant plants to 1 susceptible. In some cases there were a few plants which failed to become infected in families which, judging from the majority of the plants inoculated, should have reacted as homozygous for susceptibility. Upon reinoculation it was found that the plants really were susceptible and had merely escaped infection. In the case of the heterozygous families it is not surprising, therefore, that the number of resistant plants is a little larger than expected. Apparently, there should have been a simple ratio of 3 resistant plants to 1 susceptible. There is a deviation from the expected of  $33 \pm 9.33$ .

From these data it is very evident that the segregation for resistance and susceptibility to this one biologic form of stem rust is very simple. Many desirable types were obtained in the  $F_2$  which are homozygous for spring habit or growth and are immune from Biologic Form I.

Several  $F_2$  selections, homozygous in their reaction to Form I, were inoculated with 12 other biologic forms. The results obtained were very striking and consistent, and are presented in Table V.

TABLE V.—The reaction of Marquis, Kanred, and  $F_2$  families of the cross between Kanred and Marquis, to 13 biologic forms of stem rust

Biologic form.	Variety or hybrid family number.																
	Marquis	Kanred.	29	30	31	41	42	43	47	48	54	55	60	79	80	205	181
I	S	I	....	....	I	I	....	....	....	I	I	I	I	I	I	I	S
III	S	S	....	....	....	S	S	S	S	S	....	....	S	S	S	S	S
IX	S	I	I	I	....	I	....	....	....	....	....	I	....	....	I	I	S
XIV	R	I	I	....	....	I	I	I	I	I	....	....	....	....	I	....	....
XVII	S	I	....	....	....	....	....	....	....	....	....	....	I	....	....	I	S
XVIII	S	S	S	....	S	S	....	....	....	S	....	....	S	S	S	S	S
XIX	R	I	....	....	....	I	....	....	....	....	....	....	S	I	I	I	....
XXI	S	I	....	....	....	I	I	I	I	I	I	I	I	I	I	I	S
XXIX	S	I	I	I	I	I	....	I	....	....	....	I	I	I	I	....	....
XXXII	S	S	....	....	....	S	....	....	....	S	S	S	S	S	....	....	....
XXXIV	S	S	....	S	....	S	....	....	S	S	S	S	S	S	S	....	....
XXXVI	S	S	....	S	....	S	....	....	S	S	S	S	S	S	S	....	....
XXXVII	S	I	I	....	....	S	....	....	I	I	I	I	I	....	I	....	....

<sup>1</sup> S—Completely susceptible; I—immune; R—resistant, a type of infection intermediate between that of S and I.

It will be noticed in Table V that as far as the reaction of the two parents is concerned, the 13 biologic forms of rust may be placed into 2 groups. The first group is typified by Form I, to which Marquis is susceptible and from which Kanred is immune.

The second group is represented by Form III, to which both Marquis and Kanred are susceptible. Here it will be seen that all of the progeny are as susceptible as either parent and identical in their reaction in this respect.

The hybrid families may also be placed in two groups; (a) those whose rust reactions are similar to the Marquis parent, and (b) those that are similar to the Kanred parent. Family 41, for example, is identical with Kanred in its rust reaction to all of the forms used, and family 181 is identical with Marquis in its rust reaction to all forms used.

These results very definitely demonstrate that resistance and susceptibility to several biologic forms of stem rust may be carried either in a single genetic factor or in different factors linked in the process of segregation.

With this fact established, it has been possible to inoculate the progeny from this cross with a single biologic form of rust from which the Kanred parent is immune. By their reaction to this one form it is clear that without inoculating they will react similarly to the other biologic forms from which Kanred is immune. In this manner, numerous  $F_2$  selections have been obtained which are pure spring types immune from all of the known biologic forms of stem rust from which the Kanred parent is immune.

#### DISCUSSION OF RESULTS

The results show very clearly the complicated nature of the genetic difference between a spring and a winter variety of wheat. The results could not be explained very well on a simple monohybrid or dihybrid basis. The segregation indicates very minute differences for this character between the individual plants. While the time of heading was divided only into weekly periods, it was very evident during the progress of the experiments that the differences between the individual plants could be determined within a few days. For practical purposes and convenience in gathering the data, however, it was decided that heading periods, one week in duration, would be sufficient to indicate accurately the nature of the segregation for this character.

The results presented in Table IV show that there was no correlation between the growth-habit character and reaction to rust. The same numeric relations exist between the susceptible and resistant plants regardless of their respective times of heading. This transfer of the rust resistance of the winter parent to the progeny having the growth-habit character of the spring parent, attained the initial objective of the experiment. Several thousand rust-resistant families were obtained in the  $F_3$  and  $F_4$  which are being studied and tested for desirable agronomic characters in general.

These results are of particular interest not only in that they contribute to the solution of the general problem of breeding varieties of wheat resistant to rust, but also from the biological viewpoint. It is a common opinion that winter forms are more ancient or primitive than spring forms. Vavilov points out that this opinion is based on the fact that the so-called wild progenitors of our cereals are winter forms. Upon closer investigation of these wild species, however, he discovered the existence of spring as well as winter forms. He states (18):

As a matter of fact, spring races in natural conditions have originated as a result of hybridization of different varieties of winter plants, and, vice versa, spring varieties could give origin to winter varieties. Both kinds of plants can be obtained synthetically one from another.

The wide genetic variability which different varieties of wheat may have for growth habit explains why spring character may be dominant over winter character and vice versa. The segregation which one would expect in a cross depends upon the factors present for the growth-habit character in the parental material. One might cross two varieties of winter wheat which from all appearances seemed to be homozygous for winter habit and in the progeny obtain some plants with the spring habit of growth. It has been shown (18) that spring types have appeared in the  $F_2$  of such a cross. Likewise two spring types may be crossed and some progenies which are winter types will be obtained. This has been shown (5) in the case of two varieties of spring barley, in the  $F_3$  and  $F_4$ , of which appeared several winter forms.

In view of the preceding facts and the data presented in this paper, it is not at all surprising that various workers have drawn different conclusions regarding the dominance of spring and winter characters in wheat and other cereals. The large number of  $F_2$  selections, homozygous for heading period and varying all the way from the pure spring character to the pure winter character, demonstrates how finely the differences for growth habit may be divided. Each  $F_2$  family (or selection) with a different heading period is of a different genetic nature. And as soon as these types become fixed, one ought to be able by intercrossing the types, to produce varying degrees of dominance of spring and winter characters.

Probably one of the most important principles that this study has demonstrated is the necessity for using known biologic forms of rust in the determination of the resistance or susceptibility of any given host. Attention was called to this principle by Stakman, Levine, and Leach (15) in 1919; by Hayes, Parker, and Kurtzweil (7) in 1920; and again by Puttick (11) in 1921. It has already been pointed out that previous to the discovery of the existence of biologic forms of stem rust of wheat, the breeding of resistant varieties met with failure time and again. Simply because a variety of wheat is resistant in a given locality for a period of years, there is no assurance that it will always be resistant in that locality or in any other locality in which it may be grown. And for the same reason one can not expect to prove conclusively, in an experimental plot, the resistance of any given variety, unless it is tested for resistance against all of the rust forms which exist in the area in which it is expected to be grown later. One can readily see the difficulty of producing an artificial epidemic in the field with certainty that a number of forms are present in sufficient quantity to have equal opportunity to attack all plants. Even if this were possible in a practical way, it would bring about difficulties in the synthetic production of a resistant variety. This point was clearly demonstrated in the work from which the data presented in this paper was taken.

The  $F_2$  population was grown in the field under an artificial epidemic produced with several different biologic forms of rust. One of the parents and many of the hybrid plants were resistant to some of these biologic forms, as was proved when they were inoculated with the pure culture in the greenhouse. But in the field, all of the plants were equally susceptible, for all practical purposes. There was no method by which one could differentiate the plants one from another under this general epidemic. If a genetic study was to be made of a given number of biologic forms in their reaction on certain host plants it was evident that the work must be carried on under controlled conditions.

After the susceptible individuals have been eliminated in this manner, the general selection for desirable agronomic characters can be carried on in the field. As soon as this desired agronomic type is obtained, it will be crossed again with other varieties or selections which are resistant to other biologic forms and this process will be continued until a desirable agronomic type has been obtained which is resistant to all of the biologic forms of stem rust.

The fact that resistance and susceptibility of the host to several different forms of rust are inherited as a single genetic factor makes this cross very valuable. Kanred is known to be immune from at least 11 different biologic forms of stem rust, and, as far as tested, the immune progeny possesses all the immunity of the Kanred parent. This has further demonstrated that a variety of common wheat may be produced synthetically which will be resistant to a large number of the biologic forms of stem rust.

## SUMMARY

(1) The segregation for growth-habit characters in the progeny of a cross between a winter and a spring wheat accords with Mendelian laws. The results indicate the presence of multiple factors for this character.

(2) Homozygous types for both spring and winter characters were obtained in the  $F_3$ . In the  $F_2$ , some plants appeared which were homozygous in  $F_3$  for a heading period which was 5 weeks later than that of the Marquis (spring) parent.

(3) Kanred is an awned winter common wheat, while Marquis is an awnless spring common wheat. In the progeny of this cross there was no correlation between growth habit and presence of awns.

(4) The segregation for rust reaction to Biologic Form I in the progeny of a cross between Kanred and Marquis wheats shows a simple Mendelian ratio of approximately three immune plants to one susceptible plant. There is a clear dominance of immunity over susceptibility, since there are no intermediate types of infection with this rust form.

(5) The progeny of this cross were inoculated with several biologic forms of stem rust and show that the reaction of the host to all of these forms is inherited as a single genetic factor.

(6) In the  $F_3$  some of the hybrid families were homozygous for the spring habit of the Marquis parent and for the rust resistance of the Kanred parent.

(7) There was no correlation between the inheritance of growth habit and the manner of reaction to stem rust.

(8) These facts further demonstrate that varieties of common wheat may be produced synthetically which will be resistant to a large number of the biologic forms of stem rust, *Puccinia graminis tritici*.

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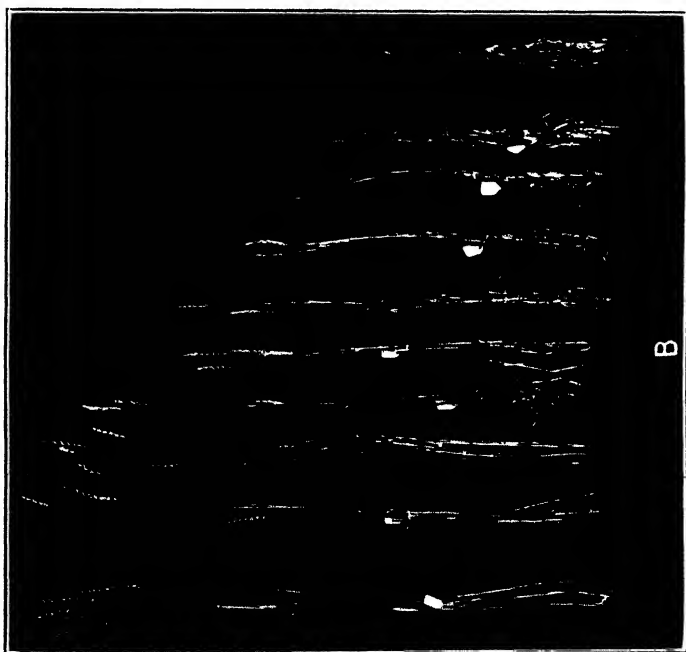
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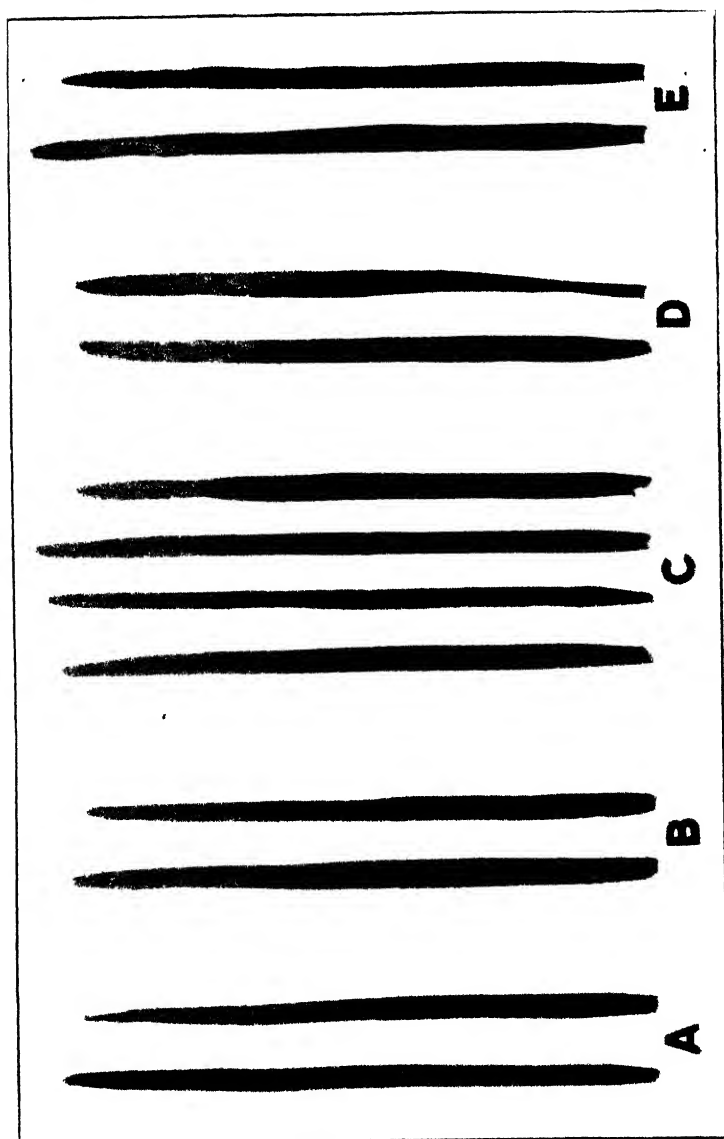


PLATE 1

A.—Showing the difference in growth habit between Kanred winter wheat (left) and Marquis spring wheat (right) at harvest time, when both are spring sown.

B.—Showing the segregation for time of heading in the  $F_2$  plants from the Marquis  $\times$  Kanred cross, when seed from  $F_2$  heading classes was spring sown. Left, Marquis; center,  $F_2$  hybrids, arranged in order of heading dates, from left to right; right, Kanred.





## PLATE 2

Seedlings of Kanred, Marquis, and  $F_3$  families of the cross between Kanred and Marquis, inoculated with a rust form from which Kanred is immune and to which Marquis is susceptible. A, Kanred, immune; B,  $F_3$  generation, immune; C,  $F_3$  generation, segregating for susceptibility to rust; D,  $F_3$  generation, susceptible, E, Marquis, susceptible.



# EFFECT OF ORGANIC DECOMPOSITION PRODUCTS FROM HIGH VEGETABLE CONTENT SOILS UPON CONCRETE DRAIN TILE<sup>1</sup>

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## PREFACE

The work which is covered in the following report was begun purely as an engineering study. It arose out of the fiduciary duty of the engineer to protect the interest of his client, namely the man who ultimately pays the bill and upon whose prosperity the permanence of the whole business rests. Necessity made the scope of the investigation much broader than was at first expected. Accident drew attention to the apparent failure of a structural material under certain limited conditions which opportunity gave a chance to investigate. This does not, however, mean that the material will fail under any other conditions than those investigated. Nor does it mean that material not investigated is therefore exempt from criticism. It is believed that this investigation very materially aided a movement for the general improvement of the material investigated, not only for limited but, also, for the general application of the material.

Other material, not covered in the scope of these investigations, but used for the same purpose, is, under certain conditions, as liable to failure as is the material investigated. A general movement for its improvement is very much to be desired. A recognition of the special adaptability of each material to its own particular class of work would aid materially in stabilizing the business.

## PART I. GENERAL INTRODUCTION

The extremely rapid development, particularly in the last few years, of the drainage of wet farm lands and the increasingly large investments in drainage that are being made by the farmers of this country are making necessary an intensive study of both materials and methods. Burned clay was the first material to be used extensively in this work, and by its use the early science of land drainage was developed.

The adaptability of concrete for making drain tile soon became obvious and the manufacture of concrete drain tile is now one of our well established industries. A very large proportion of the lands which may be profitably drained are either in large alluvial deposits or in immense plains left by glacial action. Under each of these conditions the formations which would carry good industrial clays are in many instances deeply buried and the farmer may have to go a considerable distance for his supplies, and pay heavy freight charges on them.

Another factor of very considerable importance is that the efficient handling of clay products requires a very considerable outlay for plant, necessitating, in its turn, a market, developed before the establishment of the plant and of sufficient size to keep the plant busy. This is impossible in the newer districts.

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Concrete tile, on the other hand, may be made in small and inexpensive plants, adapted to seasonal business of various sorts, and the bulk of the material used is available at a comparatively low cost.

The formation of drainage districts and the digging of large outlets is of little value without complete interior drainage of the wet lands by the construction of laterals, spaced as the soil condition may require. In outlying districts the excessive cost of clay tile due to long haul prohibits its use. With this in mind it may be said that concrete, if it can be made durable, is potentially the controlling factor in the drainage development of the newer parts of the country.

Unfortunately, when first manufactured, concrete tile made a bad start. It was claimed that as most of the water got into the tile through its walls and not at the end of the tile, the more porous those walls were, the more efficient the drain would be. This erroneous idea was honestly promoted by engineers and was used as a sales argument by the manufacturers of concrete. Standard specifications at the present time permit an absorption of 10 to 12 per cent and unscrupulous manufacturers have been making a product that merely holds together long enough to get it into the ground. Failure due to the breaking down of the tile has been common, but the firms manufacturing poor material have generally been of short life while those who turned out a good product have usually grown and increased their business. The result is that the average product of today is far superior to that of 10 or even 5 years ago.

It has frequently been noted that tile made by certain manufacturers, or, more correctly, the tile made by manufacturers in certain districts did not last as well as tile manufactured in other districts. The manufacturer was invariably blamed for any failure.

#### INVESTIGATIONS

In most average soils, well-made concrete tile appears to be permanent. As the vegetable and mineral matter of soils varies, however, the concrete tile disintegrates.

In some of the arid and semi arid districts of the West and Middle West, for instance, concrete has broken down in wet soil containing a high percentage of some alkalis, particularly alkaline sulphates. The cause is becoming fairly well understood, but the cure is still under investigation. This disintegration appears to have first been reported officially by Headden (7)<sup>2</sup> and Tannatt (14) in 1908. Since that time the subject has been very carefully investigated, notably by Bates, Phillips and Wig (2) in 1912; Wig and Williams (16) in 1915; Wig, Williams and Gates (18) in 1916; Steik (10) and Wig, Williams and Finn (17) in 1917; Steik (11) in 1919, and Miller (8) and Williams (19) in 1922.

The outstanding fact developed by this work is the destruction of concrete by alkaline sulphates of a comparatively high concentration in the soil water of alkaline soils.

Winter and Musselman (21) in 1915, and Winter (20) in 1917, working at the Michigan Agricultural College, investigated the solubility of concrete in water and in various acids.

The University of Washington has made tests covering the destruction of concrete by carbonic acid.

In Europe several marsh investigators have noted the disintegration of concrete in "Hoch moor." This corresponds to our "muskeg," which is

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 499-500.

composed almost entirely of moss and other low order plant accumulations, without inwashed soil or lime.

Vogler (15) in his "Grundlehren der Kulturtechnik," published in 1909, draws a strong distinction between low moor and high moor, repeatedly affirming the resistant quality of cement tile in low moor and its destruction in high moor.

Tacke (13), in 1910, published a paper on the substances in peat which would probably be destructive to concrete. He mentions the disintegration of concrete in a moor containing as high as 17 per cent of iron pyrite. He also discusses the probable destruction from humic acids of concrete in high moor and its permanence in low moor.

Bersch (3) in his "Handbuch der Moorkultur," published in 1909, makes the same distinction between low and high moor as does Vogler, and draws the same conclusions regarding the durability of the concrete tile in the two classes of peat.

None of these authorities followed up his observations by any published research results, but the behavior of concrete tile in peat was considered of such importance that a subcommittee of the German Committee on Reinforced Concrete was assigned to the task of making investigations. Their work was interrupted by the war.

In this country previous to 1921, if there had been any suspicion of destructive effect on concrete tile due to weak soil acids, no report had been made of any investigations induced by such a suspicion. Wig, Williams and Finn (17) in 1917, while investigating the effect of alkali on tile, noted a disintegration that took place on one of their tile lines at Columbia, Mo., where tile were laid in supposedly neutral soil as a control against the disintegration of similar tile laid in alkaline soil. They say in their report:

An exceptional condition has arisen at this project, presumably caused by some local action, in that the tile of series 1 to 16, excepting series 5 (tar coated), showed evidences of disintegration on the lower outer surface, indicated by the apparent dissolving away of cement leaving the sand grains coated with a brown stain.

The tile at Columbia were laid in mineral soil overlaid by 12 to 15 inches of black soil.

Williams (19) in 1922, reports on the same tile that in all cases except those which had been in the ground only one and two years, and those which were tar coated, the tile were stained and pitted.

Alway (1) in 1922, refers to the work of Tacke at some length. He questions the probabilities that the breaking down of the concrete tile is due to the action of organic acids or that concrete tile break down in peats containing a high percentage of lime. He says:

Humic acids are not carried in the bog water of high lime peats. These acids are more or less soluble in pure water and in weak mineral acids, as phosphoric and boric, but insoluble in hydrochloric and sulphuric acids. With soluble compounds of calcium, magnesium, iron and aluminum, they form insoluble compounds, so called humates which are insoluble in water as well as in moderately concentrated alkali solutions.

Soluble sulphates, particularly those caused by the breaking down of iron pyrite (a common condition in acid peat bogs) seem to him to be the most probable cause of disintegration, but he also includes hydrogen sulphid and alkali waters.

Stewart (12), collaborating with Doctor Alway, and writing in the same publication, makes a direct statement along the lines suggested by Doctor Alway's paper.



In February, 1921, the writer (4) offered the theory that concrete tile were liable to disintegration in any soil carrying a high percentage of organic matter. This was followed in July of the same year by a further statement (5) to the same effect.

In addition to the authorities quoted, numerous observers in the last two years have reported a breaking down of concrete tile lines in soils high in organic matter. None of the authorities quoted, however, either in this country or Europe, have followed up their observations with exhaustive research which they have later reported. Nor have any of them considered it probable that concrete tile would break down in soils carrying a high percentage of lime. Consequently in the work covered by these investigations, this point has been especially recognized and covered.

#### UNIVERSITY MARSH

A tract of 110 acres of peat marsh is located on the experimental farm of the University of Wisconsin. For the past 10 years this land has been used for experimental purposes in drainage. This area, which at one time was a swampy bay of Lake Mendota, is generally known as University Marsh. Most of its surface is a little above the lake and the soil consists of fibrous peat 2 to 6 feet deep, underlain by sand and clay. In the deeper parts of the marsh there is a bed of marl between the peat and the mineral soil below. The marl bed varies considerably in thickness up to a maximum of about 18 inches, and around its margin frequently blends with, and is interlaid by sand. Since diking, various types of drainage have been worked out, improvements and changes being made as their value was demonstrated.

On the higher side of the marsh are a number of high morainal hills from which the surface water of about 400 acres drains down. Heavy seepage also works into it from below, especially in the sandy portion. This is sufficient if the stream is concentrated into a pipe to lift the water  $2\frac{1}{2}$  feet above the marsh surface.

From the beginning, this experimental work on the marsh has been under the direction of E. R. Jones, professor of agricultural engineering at the University of Wisconsin and State drainage engineer. During the fall of 1919, and the season 1920, the work was under the direct charge of the writer, whose principal function was a determination and compilation of data on the physical change in the marsh brought about by the various stages of drainage and cultivation.

Part of the work consisted of an examination of the tile lines themselves. This was done by groups, taking as a group a series of tile having a common outlet and laid at the same time and under similar conditions. On each group several pits were dug for the purpose of examination. If no unusual conditions were disclosed, it was assumed that none had developed. If, however, any unusual condition were disclosed, more pits were dug for the purpose of determining whether or not the unusual conditions were inherent in the group.

#### DISINTEGRATED TILE

Seven lines of tile, two of which were concrete, were laid by students in May and June, 1914. Lines 5 and 7 were of concrete. In December, 1919, on opening these two lines, the tile were found to show signs of failure.

More pits were opened, and it was discovered that the entire lines were badly disintegrated, decay being greatest at the upper end and farthest away from the pump house. It was at first thought that the tile might have broken down, owing to bad workmanship, but this was disproved when it was found that at the extreme upper end of the tile lines, where rotted muck had flowed in and partially filled the tile, the tile had been protected, and had not decayed. It still bore the marks made by the mold, and, upon breaking, the interior of the tile wall appeared to be in perfect condition. The surface of the tile, both inside and outside, was rough, due to the grains of sand and gravel falling away. Inside the disintegrating layer, the tile was discolored for about  $\frac{3}{8}$  of an inch, but appeared to be dense, while a thin layer in the center of the wall was unaltered. This last fact shows that the tile had not broken down due to the passage of waters through the walls, but to solvent action upon the walls themselves. Disintegration was greatest on the outside of the tile and at the ends and much greater on the top than on the bottom. Disintegration on the inside of the tile was greater at the top than on the bottom, and was not greater at the water line, except at the joints, where it met heavy disintegration from the outside.

Since the tile showed signs of good workmanship, it was assumed that the disintegration must have been due to the solvent action of some substance present in the soil.

Correspondence was begun and the literature searched to secure information which would throw some light on the reason for the disintegration. The most probable solution of the difficulty was first presented by Prof. H. B. Roe, of the University of Minnesota, who cited the destruction of concrete tile in his State by the action of sulphate salts. This possibility was further investigated and talked over with chemists. The conclusion tentatively arrived at was that the disintegration was caused by sulphates produced by the decomposition of proteins in the peat. This solution seemed satisfactory.

Work on the marsh was discontinued at this point because of cold weather. The compilation of data could not go ahead until more field work was done and opportunity was taken to test out the assumed presence of sulphates in the marsh and their effect on new tile.

## PART II. PRELIMINARY STUDIES

### LABORATORY INVESTIGATIONS

With the advice and assistance of Prof. E. Truog and E. J. Graul of the Soils Department, laboratory studies were made, beginning in February, 1920. A cement tile company furnished new, uncontaminated tile for the tests. These tile were machine made, very dense, steam cured, and three months old at the time of the tests.

### MARSH WATERS

No sulphur in the form of sulphates could be detected in water from the marsh by the addition of barium chlorid to a hot solution slightly acid with hydrochloric acid. The assumption that the disintegration was due to the presence of sulphate salts was therefore unfounded, and the whole cause of the destruction still remained obscure.

The next step was to find actually what was in the marsh waters and if those marsh waters would dissolve the tile. Iron and aluminum were

thrown down in considerable quantity by ammonia. Lime magnesia was also thrown down by ammonium carbonate.

Lime in the water was determined by the following method: To 100 cc. of the water a few drops of HCl were added to dissolve anything which might be held in suspension, and then 5 gm. of ammonium chlorid. Ammonium hydroxid was added in sufficient quantity to make the solution strongly alkaline and acetic acid to make the solution just slightly acid. After boiling, the iron, aluminum, and phosphorus were filtered off and the precipitate carefully washed. To the boiling filtrate and washings, 10 cc. of 10 per cent solution of ammonium oxalate were added, then dilute ammonia until the solution was slightly alkaline. After digestion the solution was allowed to stand over night, then filtered on ash-free filter papers and washed with hot water.

The precipitate on the filter paper was then dissolved with 10 per cent solution of sulphuric acid. Next, 2 cc. more of  $H_2SO_4$  was added, plus enough water to bring the volume to 200 cc. The solution was then warmed and titrated with  $\frac{1}{10}$  normal solution of potassium permanganate. Lime was calculated on the basis of 1 cc. of solution (.0028 gm. of CaO). The results of these tests are given in Table I.

#### TESTS WITH CARBONIC ACID

Carbonic acid was next suspected as being the solvent. A quantity of distilled water was saturated with pure carbonic acid. Weighed fragments of tile of approximately the same shape and size and from which all loose particles were removed were placed in 500 cc. bottles and 475 cc. of the various waters added. The bottles were then tightly corked, placed in a revolving shaker machine and agitated 24 hours intermittently for 3 days. Duplicate samples of each were run. It was attempted to determine the solubility of the tile by determining the amount of lime originally in the water and in the sample run. It was found that lime was actually thrown out of solution from the waters taken from the bog and that the carbonated water did not contain nearly as much lime as did the distilled water. The results are shown in Table I.

TABLE I.—Grams of CaO per liter, in marginal and bog waters in their original condition, as collected in February, and in these and in distilled and carbonated waters after agitating 3 days with similar pieces of new tile

Test No.	Original water.		Waters agitated with tile			
	Marginal.	Bog	Distilled	Carbonated	Marginal.	Bog
	Gms	Gms	Gms	Gms	Gms	Gms
1	0.0896	0.0854	0.2156	0.1120	0.0651	0.0945
2	.1120	.0857	.2226	.0476	.0588	.0756
3	.1106	.0868	.1365	Broken.	.0525	.0357
4	.1036	.0784	..	.....	.....	.....
5	.1008	.0770	.....	.....	.....	.....
Average	.1033	.0826	.1918	.0798	.0588	.0686

In the agitated waters it will be noted that some of the tests in a series show a marked variation from others in the same series. This was attributed to the fact that the samples of tile were probably far from uniform.

Two outstanding facts were indicated. The first was a greater solubility of the tile in distilled water than in either the carbonated or bog water. The second was that though pieces of tile had been agitated for 3 days in water from the margin of the marsh, the quantity of lime actually in the water was less than in the beginning.

This result showed that the original premises were not well founded. They were either incorrect or something had been omitted from them. Within the power of the test to show it, carbonic acid was not causing the destruction of the tile; neither was the marsh water, apparently.

#### TESTS FOR ALKALI

There appeared to be no explanation for the results given in the Table I until it was suggested that perhaps the marsh waters did not actually contain free carbonic acid and that the tile, even after a lengthy curing, might contain free alkali. To test this, a quantity of tile was powdered and to it a small amount of distilled water was added which was immediately poured off and filtered. It gave a powerful reaction with phenolphthalein, showing that the tile was not only alkaline but that a considerable quantity was soluble in water. A quantity of the powdered tile was boiled in distilled water, filtered, and while still hot 100 cc. of the filtrate measured. This gave 3.582 gm. of residue on evaporation, a surprising percentage considering the age of the tile.

The marginal and bog water was then tested on the assumption that they contained free organic acids. Methyl orange showed that they did not. They were then titrated with standard sulphuric acid solution,  $\frac{1}{4}$  normal strength, using methyl orange as an indicator. The test disclosed strong alkaline reactions, showing unneutralized alkalies of the strength indicated in Table II.

TABLE II.—Showing the amount of  $\frac{1}{4}$  normal acid solution required to neutralize the free alkali in marginal and bog water and its equivalent in CaO per liter of water

Equivalents.	Marginal water	Bog water.
Cubic centimeters of one-fourteenth normal solution used	15.30	14.80
CaO equivalent per liter, gm.	.1530	.1480

The contradictory results obtained were now explained on the basis of the precipitation of calcium carbonate formed from the more soluble calcium bicarbonate by the free alkali of the tile taking away one-half of the carbonate radicle.

However, this did not make the explanation of the disintegration of the tile any clearer; instead, it became still more obscure.

#### COMPARISON OF OLD AND NEW TILE

The results of the tests using the new tile being apparently so different from what had actually happened in nature, it became necessary to compare the old and new tile in order to determine whether or not the new tile differed in any material degree from that which originally went into the drains.

One entire new tile was taken from the marsh, wiped with a towel, and weighed while still wet. It was then dried to constant weight in an electric oven at  $110^{\circ}\text{C}$ . and again weighed. The loss was 11.90 per cent, representing the proportion of water contained.

Similarly two new pieces of new tile were weighed in their natural air-dry condition, dried in an electric oven at  $110^{\circ}\text{C}$ ., weighed again, boiled for two hours in water, and weighed again. The results are tabulated in Table III.

TABLE III.—Percentage of water absorbed by old and new tile

Samples.	Weight at $110^{\circ}\text{C}$ .	Water absorbed in ground.	Water absorbed from air.	Water absorbed on boiling.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Tile from marsh	100	11.90		
New tile, sample (a)	100	.....	1.551	6.631
New tile, sample (b)	100	.....	1.545	7.095

From this table it will be seen that the tile which was taken from the marsh had nearly double the porosity of the new tile. How much this porosity was altered during the time it was buried in the ground it is impossible to say. From the maker's statement there is no doubt that the tile was less carefully made than that now turned out by the same manufacturer. An absorption of 12 per cent would indicate a good average tile at the time these were made, and is about the average of material turned out by the less careful manufacturers at the present time.

#### COMPARATIVE ALKALINITY OF TILE

The next step was to compare the alkalinity of the old and new tile. It would be natural to assume that any free alkali would be very quickly neutralized after the tile was placed in the ground. A quantity of the old tile and of the new tile were pulverized and 2 gm. taken from each. Then 200 cc. of distilled water was added to each sample and phenolphthalein used as an indicator. The solution was titrated by adding at successive intervals  $\frac{1}{4}$  normal solution of sulphuric acid, extreme care being used, particularly toward the end of the operation, not to permit the solution to contain any free acid and so expel carbonic acid. The operation was carried on over 2 days, titrating about every half hour with results as shown in Table IV.

TABLE IV.—Amounts of  $\frac{1}{4}$  normal acid solution required to neutralize free alkali in 2 gm. each of old and new tile, and percentages of free alkali indicated

Item.	Old tile.	New tile.
Grams of tile used	2.00	2.00
Cubic centimeters of $\frac{1}{4}$ $\text{H}_2\text{SO}_4$ solution used	17.70	40.50
Equivalent grams free $\text{CaO}$ or equivalent	.0354	.0810
Per cent free $\text{CaO}$ or equivalent	1.770	4.050

The results given in the preceding table are very remarkable. Not only did the new tile after a presumably thorough curing still contain 4 per cent of free alkali, but the old tile after 5 years in the ground, during which time it was partially destroyed, still contained nearly half as much. The importance of the fact indicated can hardly be overestimated. It will be discussed later.

#### SOLUTION IN HCl

New tile in concentrated HCl was then tested. Strong evolution of bubbles showed the presence of a considerable percentage of carbonate. The dried filtrate was ignited and treated again with acid. The exact percentage of solubility is shown by Table V.

TABLE V.—*Percentage of new tile soluble in concentrated HCl*

Constituent.	Percentages.
Insoluble silica	47.42
Total soluble	52.58
Soluble silica	3.40
Total solubles other than silica	49.18

#### ULTIMATE SOLUBILITY IN ORGANIC ACIDS

In order to ascertain the percentage of the tile which might be ultimately soluble in organic acids and to establish a relation between it and the aggregate used, an acid of low hydrogen-ion concentration was chosen, namely, quarter strength acetic, and samples of the old and the new tile and the aggregate from which the tile were made were treated with it. The acid used was supposed at the time to be full strength, but was later found to be about 85 per cent. From each sample previously dried in the electric oven, 5 gm. was taken and boiled for 10 minutes in the dilute acid and the residue weighed after drying in the electric oven at 110° C. The results are given in Table VI.

TABLE VI.—*Percentages of old tile, new tile, and aggregate soluble in 21 per cent strength acetic acid*

Item.	Old tile.	New tile	Aggregate.
Grams taken	5.00	5.00	5.00
Residue	3.3090	3.2777	3.5152
Loss	1.6910	1.7223	1.4848
Per cent soluble	33.82	34.45	29.69

## ANALYSIS OF AGGREGATE

A screen analysis of the sand and gravel aggregate used in the manufacture of the tile gave:

Grade:	Per Cent of Total.
Coarser than 10 mesh . . . . .	46. 6
10 to 20 mesh . . . . .	13. 4
20 to 40 mesh . . . . .	18. 2
40 to 60 mesh . . . . .	12. 2
60 to 80 mesh . . . . .	4. 8
80 to 100 mesh . . . . .	1. 6
100 mesh . . . . .	2. 4
	99. 2

Tomlinson<sup>3</sup> has calculated the mineralogic proportions in Waukesha sand by relative density and microscopic count, proportions of carbonates and of quartz in different sizes of Waukesha sand, as follows:

Group:	Carbonates.	Quartz.
Coarser than 10 mesh . . . . .	72. 1	1. 5
10 to 20 mesh . . . . .	72. 1	7. 0
20 to 40 mesh . . . . .	55. 7	35. 4
40 to 100 mesh . . . . .	36. 9	58. 1

*Proportion of Mineral groups in Waukesha sand*

Group:	Per Cent.
Igneous rock . . . . .	0. 42
Shale group . . . . .	
Quartz group . . . . .	32. 31
Dolomite group . . . . .	65. 37
Feldspar . . . . .	
Heavy minerals . . . . .	. 83
Not sorted . . . . .	1. 07
	100. 00

## TESTS OF CEMENT

Both the old and the new tile had been made with a slag Portland cement of average chemical composition. This cement was also treated with acetic acid to ascertain its solubility. It showed a trace of carbonic acid bubbles.

A quantity of the cement was mixed into a thick paste with water, thoroughly worked and rolled into balls by hand, then flattened on glass, made into patties and allowed to set one day under a moist cloth. At the end of that time, the patties had set firmly and showed no signs of malformation, swelling, or shrinkage cracks. Boiled one hour in water, they showed no signs of failure.

Some of the patties were allowed to set three days in water and at the end of that time appeared to be in perfect condition. They were then powdered, and 2 gm. dried at 110 C°, were treated with 21 per cent strength acetic acid. Other patties, made in a similar way at Minnesota, were allowed to set in water for three months, and then five months in air at a temperature averaging 75° F. These were powdered and treated like the others. The first test showed no evolution of bubbles, but the second gave bubbles in considerable amount, showing that the cement had carbonated during the eight months set. The result of the two tests are tabulated in Table VII.

<sup>3</sup> TOMLINSON, C. W. UNPUBLISHED THESIS. Univ. of Wis., Col. of Engin., 1915.

TABLE VII.—Solubility of cement set 4 days and 8 months in acetic acid of 21 per cent strength

Item	4-day set	8-month set
Weight of sample (gm.)	2	2
Weight of residue (gm.) { (a) . . . . .	. 559	{ 0. 4290
(b) . . . . .		
Percentage of solubility { (a) . . . . .	72. 02	{ 78. 55
(b) . . . . .		

The two tests show an average increase in solubility of 5.50 per cent. If this is to be explained on the basis of calcium hydroxide becoming carbonated during the eight months it would indicate a proportion of unneutralized lime in the freshly set cement equal to 25.8 per cent.

## ANALYSIS OF CEMENT

A cement company very kindly furnished the following analysis of their cement and a report of physical tests.

*Chemical analysis of cement, average of daily analyses, March, 1920*

	Per cent.
Silica (SiO <sub>2</sub> )	22. 04
Oxid of Iron (Fe <sub>2</sub> O <sub>3</sub> )	3. 30
Alumina (Al <sub>2</sub> O <sub>3</sub> )	5. 70
Lime (CaO)	62. 64
Magnesia (MgO)	3. 73
Sulphuric Acid (SO <sub>3</sub> )	1. 23
Loss on Ignition	. 50

*Physical tests of the same cement, average for February, 1920*

Fineness.	
Passing 200-mesh sieve	81 per cent.
Setting time:	
Initial set.	2 hrs. 45 min.
Final set	5 hrs. 5 min.
Tensile strength:	
1 part cement to 3 parts standard Ottawa sand—	
3 days (1 day in moist air and 2 days in water)	239 lbs
7 days (1 day in moist air and 6 days in water)	322 lbs
28 days (1 day in moist air and 27 days in water)	420 lbs

## RESULTS OF PRELIMINARY STUDIES

Up to this point the work done may be considered as preliminary studies, though that was not the intention when some of the tests were begun. The principal points brought out may be enumerated as follows:

- (1) The alkalinity of the marginal and bog waters in February.
- (2) The powerful alkalinity of the tile even after 5 years in the drain.
- (3) The similarity of the old and new tile except in the matter of density.
- (4) The solubility of the thoroughly matured tile in weak organic acid.
- (5) The absorption of CO<sub>2</sub> during long a period of curing.



## Analyses of American Portland Cements (from "Portland Cement")

Made from—	Where made.	SiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	CaO	MgO	K <sub>2</sub> O	Na <sub>2</sub> O	SO <sub>3</sub>	Loss.
Cement rock and limestone.	Nazareth, Pa. ....	19.92	2.28	7.52	62.48	3.79	0.52	0.66	1.51	1.46
	do .....	21.14	2.30	6.94	63.24	3.26	.36	.51	1.12	1.24
	Bath, Pa. ....	19.64	2.80	7.52	62.31	3.04	n. d.		1.60	1.48
	Alpha, N. J. ....	21.82	2.51	8.03	62.19	2.71	n. d.		1.02	1.05
	Northampton, Pa. ....	21.94	2.37	6.87	60.25	2.78	.61	.87	1.38	3.55
	Coplay, Pa. ....	22.26	2.10	5.36	63.32	3.81	n. d.		.89	1.24
	Osmrod, Pa. ....	22.20	2.27	6.69	62.61	3.00	.32	.61	1.32	1.56
	Martins Creek, Pa. ....	20.23	2.50	7.12	62.94	3.38	n. d.		1.45	1.25
	Reading, Pa. ....	24.16	1.45	5.10	62.95	3.12	.21	.50	1.35	1.40
	Ban City, Mich. ....	20.72	2.85	7.17	62.64	1.97	.48	.12	1.42	2.58
Limestone and clay or shale.	Wellestone, Ohio .....	21.84	5.05	6.77	62.66	.80	n. d.		1.24	
	Chanute, Kans. ....	20.74	3.72	7.06	62.76	1.78	.41	.23	1.12	1.40
	Ada, Okla. ....	12.28	3.20	6.36	59.66	3.11	.80	.25	1.40	2.82
	*Stroh, Ind. ....	21.78	2.65	7.31	62.35	2.88	0.47		1.78	.78
	*Glens Falls, N. Y. ....	21.50		10.50	63.50	1.80	.40		1.50	n. d.
	Alsen, N. Y. ....	23.94	3.20	5.62	62.32	1.77	n. d.		.90	1.68
	Fordwick, Va. ....	21.31	2.81	6.54	63.01	2.71	n. d.		1.42	2.01
	Davenport, Calif. ....	25.38	1.20	3.34	62.96	1.30	n. d.		.35	4.58
	Cement, Calif. ....	22.34	3.30	7.00	60.72	1.20	n. d.		1.05	2.54
	*Baker, Wash. ....	24.63		8.50	62.88	1.60	n. d.		1.33	n. d.
Marl and clay	St. Louis, Mo. ....	23.12	2.49	6.18	63.47	.88	n. d.		1.34	1.81
	Demopolis, Ala. ....	19.36	4.10	9.18	63.20	1.16	n. d.		1.18	1.12
	*Portland, Colo. ....	21.88	2.85	7.14	64.94	Trace.	1.18		.73	1.08
	*Middlebranch, Ohio .....	21.24	4.14	7.85	63.22	.28	.68		1.11	1.32
	*Coldwater, Mich. ....	21.22	3.83	7.51	63.75	.82	n. d.		1.58	1.02
	Sandusky, Ohio .....	21.93	2.35	5.99	62.92	1.10	.63	.27	1.55	2.92
	*Bronson, Mich. ....	22.90	3.60	6.80	63.90	.70	1.10		.40	.60
	*Harper, Ohio .....	21.30	2.00	6.95	62.50	1.20	n. d.		.68	4.62
	*Warners, N. Y. ....	22.04	3.41	6.45	60.92	3.53	n. d.		1.25	...
	Chicago, Ill. ....	22.41	2.51	8.12	62.01	1.68	n. d.		1.40	1.08
Limestone and blast furnace slag.	do .....	23.06	2.88	8.16	62.10	1.88	.36	.58	1.57	...

<sup>1</sup> Analyses made by Richard K. Meade, with the exception of those designated \*.

The average is represented by the following:	Per cent.
Silica. . . . .	22.0
Alumina. . . . .	7.5
Iron oxid*. . . . .	2.5
Lime. . . . .	62.5
Magnesia. . . . .	2.5
Sulphur trioxid.. . . .	1.5

## PART III. DEFINITE RESULTS

In the preliminary work where tests were made with tile, the proportion of tile to water was far in excess of the proportion between the tile in a marsh, and the marsh itself was always sufficiently high to make the combined solution strongly alkaline. In all later work the aim was to reverse the proportions so as to make the influence of the marsh predominate. The marsh water, too, taken in the month of February, was alkaline. It became necessary to determine if the water in the marsh remained alkaline under the influence of warmth and if the tile were acted upon by a large excess of solutions produced under warm conditions.

## TEST WITH SURPLUS OF BOG WATER

The results of the tests with a comparatively large piece of tile in a small quantity of water being unsatisfactory, due to the excessive alkalinity of the tile, another scheme was devised which was intended to subject tile to a large quantity of water. In order to increase the rapidity of action, the surface of the tile was increased as much as possible by powdering the tile. From this powdered material 50 gm.

was placed in a 1-inch glass tube, 10 inches long, stoppered at each end, a glass tube giving ingress and egress at bottom and top. The inlet tube at the bottom was curved downward on the inner end to prevent the tile grains from escaping downward through the tube. Peat taken from a depth of 5 feet in the same pit from which the bog water was taken was mixed with distilled water in a 2-gallon glazed earthen jar having an orifice near the bottom. A glass tube led through this orifice and connected on the inside with a long inverted "U" on the bottom of which was a glass funnel covered with muslin as a filter. The tube through the orifice connected on the outside with a filter made of a 1-inch stoppered glass tube in which was asbestos pulp packed above glass wool. The object of the filter was to prevent the escape of any trace of solid matter into the powdered tile. At the upper end, the tube containing the powdered tile connected with a glass tube which led downward into another weighed filter made of a porcelain "gooch" in which  $\frac{1}{8}$ -inch of shredded asbestos had been packed. A pump was connected on below the "gooch" to draw the water through the system from the mixture of peat and water in the earthen jar. The last filter was carefully weighed after drying it in an electric oven at a temperature of  $110^{\circ}$  C. In action the pump sucked the water from the jar through the first filter, through the powdered tile, and through the second filter, which caught all fine sediments carried upward by the descending current. It was the intention to weigh the filter and the contents of the tube to ascertain the percentage of loss in weight, if any.

#### SETTING OF POWDERED TILE

The experiment as outlined was a failure, but several most interesting facts were discovered. At the end of the first day the apparatus began to work badly and more force had to be put on the pump to draw the water through. At the end of two days, the apparatus refused to work at all. It was taken apart and in the "gooch" was found a considerable thickness of brown semigelatinous rubbery mass completely clogging it. Where the water from the peat had passed upward through the powdered tile, that also had been stained brown. The "gooch" was disconnected and the water from the tile was led downward through a long tube acting as a siphon into a large glass container. At the end of another 24 hours the flow had entirely stopped and the powdered tile had "set" firmly.

A sample of powdered tile was moistened with water and allowed to stand overnight, and found to be set firmly in the morning. The same was done with the old tile, powdered, with like result, though the set was not so strong. This was repeated with both tile, using a large excess of carbonated water, the powdered tile being shaken up in the water. Both, on settling, set much more strongly than before.

#### 10-DAY PEAT CULTURES

To overcome this difficulty of the powdered tile "setting" another scheme was devised. Peat from the top of the University Marsh, which gave a slightly acid reaction, peat from the pit, which was alkaline, and peat from north central Wisconsin, which was strongly acid, were placed in glazed earthen jars and distilled water added. The jars were then allowed to stand 10 days in a warm room and the water tested for acidity. All were acid, and in the following proportions when titrated with  $\frac{1}{4}$  normal hydroxid solution, using phenolphthalein as an indicator.

TABLE VIII.—Showing amount of  $\frac{1}{4}$  normal alkaline solution required to neutralize acidity of 100 cc. water from 10-day cultures of surface and deep peat from University Marsh, and northern acid peat; also its equivalent in grams of free lime per liter

Water sample (10-day culture).	$\frac{1}{4}$ normal solution used.	Equivalent per liter to CaO.
	cc.	Gm.
Surface peat (a) . . . . .	0.55	0.0110
Do (b) . . . . .	.60	.0120
5-inch peat (a) . . . . .	.45	.0090
Do (b) . . . . .	.50	.0100
Northern acid peat (a) . . . . .	6.75	.1350
Do (b) . . . . .	6.80	.1360

## TESTS WITH 10-DAY CULTURES

Duplicate bottles were prepared containing 2 gm. each of new tile and to each was added 400 cc. of distilled water. The same was done with carbonated water, marginal water, bog water, surface, deep peat, and northern peat culture. These were immediately tightly stoppered, placed in a shaker machine, and agitated continuously 54 hours.

It will be noted that only the northern peat water was in sufficient quantity to neutralize the free alkali in the 2 gm. of tile. This was further indicated by the rate of settling of the solids held in suspension. While all the other bottles, even the carbonated water, settled overnight, the acid peat water was still turbid after 3 days and showed distinct layers of colloidal material slowly settling in the liquid above the sediment in the bottom.

Hall (6) has shown that the alkali earth, particularly the carbonates, have a strong flocculating action assisting in the deposition of sediments. The results just obtained indicate that the products of organic decay have an even more powerful effect in retaining them in suspension.

TABLE IX.—Residue left after treating 2 gm. of tile with 400 cc. of water as in Tables I and VIII (agitated 54 hours and settled 14 hours)

400 cc. of—	Weight of tile used.	Weight of residue.	Soluble.	Increase.
	Gm.	Gm.	Per cent.	Per cent.
Distilled water (a) . . . . .	2	1.8796	6.02	.....
Do (b) . . . . .	2	1.8532	7.34	.....
Carbonated water (a) . . . . .	2	1.8983	5.08	.....
Do (b) . . . . .	2	1.8727	6.36	.....
Winter waters:				
Marginal water (a) . . . . .	2	2.0270	.....	1.35
Do (b) . . . . .	2	2.002	.....	.01
Bog water (a) . . . . .	2	2.0100	.....	.50
Do (b) . . . . .	2	2.0119	.....	.559
10-day cultures:				
Surface peat (a) . . . . .	2	2.0965	.....	4.82
Do (b) . . . . .	2	2.0960	.....	4.80
Peat 5 feet deep (a) . . . . .	2	2.0090	.....	.45
Do (b) . . . . .	2	1.8948	5.26	.....
Northern peat <sup>1</sup> (a) . . . . .	2	2.2007	.....	10.03
Do (b) . . . . .	2	2.0885	.....	4.12

<sup>1</sup> The duplicates of the last two pairs showed distinct differences in the color of their filtrates; the darker filtrate passing through much more rapidly, possibly due to a thinner filter, and carrying colloidal material with it.

## SOLUBILITY OF TILE IN DECARBONATED PEAT WATER

Next, 400 cc. of the most acid peat water was taken, from which all carbonic acid had been removed by drawing through it air passed through sodium hydroxid, and 2 gm. of powdered tile were added. The solution was then agitated in the shaker machine, the residue filtered out on a dried, weighed, ashless filter, dried in an electric oven to constant dryness, weighed, and then residue and filter agitated for 14 hours in 400 cc. of carbonated water.

TABLE X.—Results of agitating 2 gm. of tile with acid peat water and treating the residue with carbonic acid water <sup>1</sup>

Item.	Weight after treating with acid peat water	Weight of residue treated with carbonated water.
Original sample . . . . . (gm.)	2. 0000	2. 1288
Residue . . . . . (gm.)	2. 1288	1. 9129
Per cent increase . . . . .	6. 44	. . . . .
Per cent decrease from sample treated with peat water . . . . .	. . . . .	10. 14
Per cent decrease from original . . . . .	. . . . .	4. 25

<sup>1</sup> The filtering of the solution of tile with peat water was carried on with considerable difficulty, owing to the colloidal nature of the products, yet the solution showed only a slight turbidity.

## INCREASING ACIDITY OF ROTTING PEAT

In order to ascertain whether the process of decay increased the percentage of acid in solution or not, the acidity of the water on the peat cultures was tested after 20 days and compared with the 10-day culture. Conditions in the northern peat culture had been altered by the addition of more water, so that was excluded from the calculations.

A titration of 100 cc. of the water with  $\frac{1}{10}$  normal solution of sodium hydroxid was made, using phenolphthalein as an indicator.

TABLE XI.—Amount of  $\frac{1}{10}$  normal alkaline solution required to neutralize the acidity of 100 cc. of water from 20-day cultures of surface peat and deep peat from University Marsh and the equivalent in grams of free lime per liter

Sample taken.	Amount of $\frac{1}{10}$ solution used.	Equivalent to CaO per liter.
	cc	Gm.
Surface peat (a) . . . . .	2. 95	0. 0590
Do (b) . . . . .	3. 15	0. 0630
5 feet peat (a) . . . . .	3. 50	0. 0700
Do (b) . . . . .	3. 30	0. 0660

TABLE XII.—*Summary of Tables X and XI, showing acidity of 10-day cultures equivalent to grams of free lime per liter of water*

Sample taken.	Original state.	CaO equivalent.	
		10-day culture.	20-day culture.
Surface peat (a) . . .	Faintly acid. . . . .	0.0110	0.0590
Do (b) . . . . .	do . . . . .	0.0120	0.0630
Peat 5 feet deep (a) . .	Alkaline . . . . .	0.0090	0.0700
Do (b) . . . . .	do . . . . .	0.0100	0.0660
Northern peat (a) . .	Acid . . . . .	0.1350	0.1350
Do (b) . . . . .	do . . . . .	0.1360	0.1360

During the late winter of 1922, with the assistance of Mr. R. C. Reck, chemist of the drainage laboratory, University Farm, St. Paul, a series of determinations were run, using peat solutions derived from Coon Creek peat, which was alkaline. Distilled water was put on a quantity of the deeper peat from the Coon Creek experimental tracts. The water was tested one-half hour after being put on the peat, again at 10 days, and again after 30 days, the 30-day test water being free from carbonic acid. The results of the tests, in which methylorange was used as an indicator, are given in Table XIII.

TABLE XIII.—*Determinations of relative alkalinity or acidity of water from Coon Creek peat at different ages*

Age of test water.	Test No.	Amount taken.	N <sub>2</sub> O solution used.	CaO equivalent per liter.
1st day . . . . .	1	cc. 25	Gm. 0.52	Gm. 0.0292
Do . . . . .	2	50	1.04	0.0292
10th day . . . . .	1	Neut.	0.0000	0.0000
Do . . . . .	2	Neut.	0.0000	0.0000
30th day . . . . .	1	25	.40	.0224
Do . . . . .	2	25	.35	.0196

In order to ascertain if any effect were produced on the concrete tile by a weak solution of organic acid free from carbonic acid alternating with carbonic acid, 2 liters of the peat solution were taken and air passed through for 24 hours. The air was first drawn through moist sodium hydroxid to eliminate CO<sub>2</sub>. The water was then tested for acid, with results given in Table XIII. Two flasks were then taken and 400 cc. of the peat water placed in each. One gram of oven-dry, powdered tile was then added to each bottle. The bottles were then corked and agitated for 48 hours. The solutions were then filtered on weighed ashless filters and the residues dried and weighed. The residues, which were not removed from the filter, were replaced in the flasks and to each was added 400 cc. of distilled water containing pure carbonic acid. This carbonic acid water was tested and found to be 0.016 normal strength. The solutions were then agitated for 24 hours and the residue dried and weighed. The residue was then treated again with peat water and carbonic acid water, agitating 24 hours. The results are tabulated in Table XIV.

TABLE XIV.—Effect on concrete by treating repeatedly with weak peat water and with CO<sub>2</sub> water

Item.	Sample 1.	Sample 2.
Original weight (gm.) . . . . .	1.0000	1.0000
Residue after treatment with 400 c. c. of peat water (gm.) . . . . .	.9684	1.0464
Residue after treatment with 400 c. c. of CO <sub>2</sub> water of 0.016 normal strength (gm.) . . . . .	.8367	.9120
Residue after second treatment with peat water (gm.) . . . . .	.7879	.9167
Residue after second treatment with CO <sub>2</sub> water (gm.) . . . . .	.7843	.8890
Total net loss (gm.) . . . . .	.2157	.1110
Total net loss (per cent) . . . . .	21.5700	11.1000

These experiments gave the first really constructive information. They showed that the powdered tile was powerfully acted upon by the peat waters, the amount of action depending upon the acidity of the peat water. In this action a semigelatinous mass was precipitated out of the solution, increasing the weight of the total solids. On treating with carbonic acid water, this gelatinous mass was removed and about as much tile dissolved as would have been the case had the tile not been previously treated with peat water.

#### ACTION ON NEAT CEMENT

In order to ascertain if the decaying peat had any action on "neat" cement a small quantity of the same cement as used in Table VII was mixed with water to make a stiff mortar. With this mortar small patties were made by first rolling the mortar into a ball by hand and working it thoroughly together. These balls were then flattened on glass into patties which were 2 inches across, one-half inch thick in the middle, and tapering to a thin edge. The upper surface was troweled with a spatula until the "laitance" was brought to the surface. The patties were then allowed to set in warm water for a week.

After removal from the glass the patties were placed in a jar of peat and kept moist for three months in a warm place. At the end of that time the patties were broken and the fracture examined. It was found that on the lower side where the cement had been against the glass the peat solution had penetrated to about three thirty-seconds of an inch and the surface could be easily scratched with a knife. On the upper side, however, which had been packed by hand and then troweled, there was no sign of any acid penetration, and the surface was very hard and dense.

There was no experimental work done to determine whether the resistant quality of the cement was due to the greater density of the mass, to the bringing to the surface of the "laitance," or to some other cause not indicated.

#### RESULTS

In addition to the five principal points brought out in the preliminary studies, five additional ones are indicated in the later work. They are:

(6) The setting of the partially decomposed tile on grinding and moistening.

(7) The gelatinous compounds of organic matter and concrete.

- (8) The solubility of this compound in carbonic acid.
- (9) The action of the organic compounds on neat cement.
- (10) The increase in quantity of acids as decomposition proceeds.

#### PART IV. FIELD WORK

Before drawing a definite conclusion from the results of these laboratory investigations it was decided to make most careful field observations to ascertain if the laboratory findings were borne out by actual conditions. These observations have covered two years and during that time the subject has attracted considerable public attention. Reports have come in, notably from the muck lands of the South, which considerably broaden the scope of the investigations.

#### REPRESENTATIVE LOCALITIES

The field work was planned so that it should cover the greatest possible range of soil conditions. No attempt was made to get mere numbers of observations. On the other hand, an attempt was made to investigate the peats overlying dissimilar geological formations, first in Wisconsin and later in Minnesota.

#### LIMESTONE SOIL

In Wisconsin, eastern Dane County was taken as typical of the glaciated limestone country. This includes University Marsh, where the peat was underlain by marl. This has been described earlier in this paper. During 1920 a considerable amount of the tile in the eastern part of the marsh was relaid at a greater depth. Among these were alternate lines of Group VII. They included the lines opened up in 1919 from which the samples were taken. It was found that about half the tile were either collapsed or were not fit to be put back in the ground. This is well illustrated in Plate 3, A. This rapid final collapse, after disintegration was once well under way, later proved to be characteristic.

A large marsh of the alkaline type south of Madison, Wis., was also investigated. It lay in the bottom of a stream valley and in its natural condition would be subject to more or less periodic overflow and seepage and wash would be heavy. Water would either be standing or running in the tile the greater part of the time. A portion of this marsh was drained in 1914. The tile were examined in the spring of 1920. A pile of unused tile showed that the quality was not of the highest, as judged by present standards, but was a very good average at the time it was made. On opening up the tile lines an interesting condition was disclosed. At the outlet of the lines fresh drainage water did not enter the tiles in large quantities but found its outlet into the main ditch. The inside of the tile was covered with sediment and the concrete thereby protected. Under these conditions there was little disintegration. Farther up the lines, where water either stood or ran a larger part of the season, the cement of the tile had almost completely disappeared. On account of their fragility, there was considerable difficulty in taking out samples. The tile apparently retained their perfect shape and were functioning quite properly as long as they were not disturbed. The act of digging, however, caused their complete collapse. At the upper

end of the line, where the lower part of the tile was entirely protected by soft mineral soil, little disintegration had taken place, but the upper part was badly pitted where peat had lain against it (Pl. 2, A).

#### RECENT LIME DRIFT

Walworth and Ozaukee Counties were taken as representative of the heavy limestone drift area of recent formation and rich in lime. The peat swamps in this area are very narrow, have been subject to heavy wash, and are high in mineral matter. The soils are comparatively tight clays and loams and seepage is negligible. Consequently, the ground water in summer descends below the tile lines and, except for short intervals, usually remains there throughout the growing season (Pl. 2, B). Tile No. 2 came from near the mouth of a short drain where it was not subjected to standing water. No. 3, however, came from opposite the mouth of a low draw where there would be considerable seepage from the surrounding highland. The peat areas in this formation are comparatively small, and as the rate of disintegration is slow it is not expected that it will cause serious difficulty.

#### OLD RESIDUAL SOILS

Richland County was taken as representative of the unglaciated area, and here it was found that in the acid residual soils the tile broke down with comparative rapidity.

#### ACID SANDY SOILS

Wood and Juneau Counties were taken as representative of the sandstone area. These soils are for the most part very strongly acid. In this district concrete tile set in peat broke down within a year after they were laid. The sewer tile shown in Plate 4, B, was laid in clay soil high in lime. The outside gave no sign of disintegration but the inside was somewhat pitted. This area appeared to be the most destructive to concrete of any that were investigated. Throughout this whole sandy district the use of concrete in marshes appears to be only a temporary expedient. Opportunity was offered to examine the concrete culverts on the roads through the cranberry marshes of Wood County. Conditions here offer the most severe test that could be devised, for the acid waters of the cranberry marshes and reservoirs remain against the concrete throughout the season. All of the culverts and bridges examined showed signs of disintegration, the destruction having penetrated into the solid concrete. Plate 3, B, shows this very strongly.

#### GRANITE SOILS

Waupaca County was considered typical of the granite soils. An observation made at Wyanwega was extremely interesting, for the tile were laid not in peat, but in sand underlying muck. An area of some 53 acres was tiled in 1915. In 1917 the tile system began to give trouble. In 1918 several breakdowns occurred, owing to the complete collapse of the tile at those points. In 1919 there were over 20 breakdowns, one of which was about 100 feet long. Strange to say, in spite of its thicker wall, the 8-inch outlet main seemed to suffer the most. Plate 4, A, shows samples from this system. In no case except No. 4 did the peat come in contact with the tile. No. 3 was taken from a tile line on a flat piece of high ground. There was no peat on this line.



## MINNESOTA CONDITIONS

In the late fall of 1920, the writer removed to Minnesota. This State does not have drained peat bogs or swamps on the wide range of soils that occur in Wisconsin. Practically all of the Minnesota peats that have been drained are of the "high lime" type. A large proportion of them are in sloughs and depressions of the low ground of the northwestern glacial drift whose soil is composed almost entirely of ground-up shale and limestone. The peats themselves are built up mostly of plants of a high botanical order.

The low lime peats of Minnesota lie largely to the north and northeast overlying the more acid, northeastern drift and the rock outcrops of that part of the State. They are derived mostly from the remains of plants of a low order, in which the mosses predominate. Low lime peats in Minnesota have not yet proved their economic importance. Underdrainage of any soils is not far advanced in the northeastern part of the State and the farming of peat can be said to have not yet begun.

## GRAND RAPIDS STATION

## LOW LIME PEAT

Opportunity was offered to investigate only one tract in Minnesota where a low lime bog had been drained for a number of years. In fact, it is the only one known in the State where concrete tile has been used. This was on the State experimental farm at Grand Rapids, Minn. The tract was tiled in 1910 under the direction of the State experiment station at St. Paul, Minn., Prof. J. T. Stewart being directly in charge of the work. The tile lines were laid an average of about  $3\frac{1}{2}$  to 4 feet deep. Part of the area tiled was cultivated experimentally. The rest was left in its natural condition. In 1918 the system showed signs that it was not functioning properly. Water did not drain away after storms as it should. In 1918 almost the entire system was taken up and relaid. It was found that about one-fourth of the tile had completely collapsed or were not fit to put back in the ground. The design of the system was somewhat altered. The 75 per cent of the tile that were in fair condition were used a second time and the balance was replaced with clay tile.

Plate 5, A, B, and C, shows different views of one of the better tile that was stock piled and kept for use. Disintegration was not serious on the outside at top and bottom, but was considerable on the sides. The top inside shows no signs of disintegration, but the bottom inside is very badly eaten.

In June, 1921, the lines were examined. Plate 6, A, shows the specimens taken. No. 1 was from the cultivated area on the central line which was relaid. The specimen came from 50 feet south of the fence. No. 2 was taken from the same line 50 feet north of the fence in uncultivated peat. It is interesting to note the greater disintegration of the tile in the raw peat. The peat at the Grand Rapids station is very fibrous and was cut out by the tilters in large pieces. When these were thrown back into the trench they frequently formed an arch over parts of the tile without touching it. The results of this are shown in No. 2, Plate 6, A, and the actual conditions in the ground in Plate 5, D. On all of the body of the marsh the ground water would drop below the tile lines throughout the greater part of the season. No. 3, Plate 6, A, was taken from mineral soil in a part of a line that was not relaid. This was

a seepage line that ran along the western edge of the bog but, at the place where the sample was taken, cut across a point of high ground about 200 feet wide on which there was no peat.

Crumbs were broken off by hand from the rotted portion of all of these tile, powdered, shaken up with distilled water for one minute and tested for free alkali. All gave a violent alkaline reaction.

The strength and absorption of these tile were tested in the drainage laboratory of the Minnesota Experiment Station by D. G. Miller, senior drainage engineer of the United States Department of Agriculture, and J. A. Wise of the Minnesota Experiment Station. The difference in porosity between the decayed tile and that which was not decayed is not strongly brought out. The fact is indicated that the tile were of high grade for the time they were made.

TABLE XV.—Results of physical tests of concrete tile in peat (11 years) at Grand Rapids, Minn.

Item.	No. 1.	No. 2.	No. 3.
Internal diameter (in.) .	5	5	6
Weight (lbs.) .	8 72	8. 81	12. 02
Breaking load pounds (per linear ft.)	819	783	851
Absorption, bone dry and boiling method (per cent)			
Top in mold, lower side in marsh	11 8	13 6	11 7
Center piece, upper side in marsh	12 0		10. 2
Center piece, side in marsh		13 7	
Bottom in mold, upper side in marsh	11 3	11 9	
Bottom in mold, lower side in marsh		...	10. 5

#### COON CREEK HIGH LIME PEAT

Early in 1921 a movement was started to show that concrete tile would not disintegrate in peats carrying a high percentage of lime. This claim was supported by most eminent authorities and there was no published work to contradict it. As the high lime peats include all the peat areas of Minnesota that are yet of economic importance and the greater part of those of southern Wisconsin, the point raised was paramount. For the investigators to be able to limit the destruction of concrete to the low-lime peats would be of immense benefit to the States affected, for it would reduce the problem to the proportions of an academic study. It would, however, necessitate a classification of the peat areas before drainage. The results of the observations on high-lime peat are therefore given in some detail.

A peat area was selected that was assumed to offer ideal conditions for the preservation of the tile, i. e., the Coon Creek experimental tract. Here a peat area 3 to 6 feet deep is underlain in its deeper portion with marl of unknown depth and extent but sounded to a thickness of 17 feet.

The tract was selected by Dr. F. J. Alway, chief of the soils division of the Minnesota Agricultural Experiment Station, for experimental work in the agricultural utilization of high lime peats and is maintained for that purpose. On the south, the tract is bounded by low hills of wind-blown sand, derived from the limestone drift of the northwest glaciation. This sand extends out underneath the peat some 300 to 400 feet, where it drops off quite sharply, marl occupying the depression between the sand and the peat above. The part under cultivation extends a little beyond the shoulder where the sand drops away, and the outer lines of tile (laterals

1 and 2 on the map, 1 to the westward and 2 to the eastward of the main) have a few inches of marl under them below the peat.

There is very heavy seepage from the sand hills down through the sand underlying the peat. Natural escape of the seepage water at the main channel of Coon Creek is blocked by the comparatively impermeable marl. The main outlet drain of the tract cuts across the marsh and supplies the necessary outlet. The laterals at the time they were laid were from  $3\frac{1}{2}$  to 4 feet deep, and are now 3 to  $3\frac{1}{2}$  feet below the surface of the peat. There is from 1 to  $1\frac{1}{2}$  feet of peat between the bottom of laterals 1 and 2 and the sand. The main outlet, however, since it was first dug has always been about a foot below the outlets of the laterals. There has been a tendency, therefore, in times of little flow for the seepage water to escape directly from the sand into the main outlet without passing upward into the laterals. This condition would be accentuated both at the lower end of the laterals near the main and at the upper end of the laterals where their grade carries them above the almost flat ground water table. Lateral 1 carries heavy seepage from a bay indenting the high land, and the tendency for the ends of the lateral to get above the ground water level would be partially neutralized. In lateral 2, however, the tendency would be most marked at all low water stages.

The tile system was laid out and installed in 1918 under the direction of Mr. H. B. Roe, drainage engineer of the experiment station. Alternate lines were of clay and concrete. When the concrete tile arrived they were freshly cured and did not meet the strength requirements. As they also appeared to be quite uniformly porous the entire lot was condemned by Mr. Roe. Another test of the tile was made 13 days after the first, which indicated a much greater strength. Under the circumstances it was decided by Doctor Alway that it would be better to use the tile than lose a season. The tile were therefore installed.

TABLE XVI.—Tests of Coon Creek tile Oct. 2, 1918, one week after delivery (5 and 6 inch tile tested)

Test No.	Wet weight.	3-point breaking load.	Calculated breaking load.
	Lbs.	Lbs.	Lbs.
1 . . . . .	9 $\frac{3}{4}$	375	562
2 . . . . .	10	375	562
3 . . . . .	10 $\frac{1}{4}$	413	620
4 . . . . .	10	325	488
5 . . . . .	10 $\frac{1}{8}$	425	638
Average . . . . .	10.025	382.6	574

TABLE XVII.—Tests of Coon Creek tile Oct. 15, 1918, three weeks after delivery (5-inch tile tested)

Test No.	Dry weight.	3-point breaking load.	Calculated breaking load.
	Lbs.	Lbs.	Lbs.
1 . . . . .	8 $\frac{3}{4}$	538	807
2 . . . . .	8 $\frac{3}{4}$	675	1,013
Average . . . . .	8.56	606.5	910

From Tables XVI and XVII it would appear that the average cold absorption of the air tile was approximately 1.465 pounds, or 17.1 per cent, and that Mr. Roe's condemnation of the tile was well founded.

In June, 1921, an inspection of the tile was arranged for, to take place, on August 1, Doctor Alway acting as intermediary. There were present the local manager of a cement association, the director of the publicity department of the same cement association, the engineer for a Minnesota concrete pipe and tile association, Dr. F. J. Alway, of the soils department of the agricultural engineering branch of the Minnesota Experiment Station, and the writer.

Altogether, five pits were opened, the location of the first three being chosen by the representative of the cement association and the location of the last two being chosen by the writer. The first three pits were all opened on lateral 2 at 265, 450, and 600 feet from the main. This lateral, as previously described, is underlain by marl, at a depth of about 1 foot below the grade of the tile. Two tiles were taken from each pit and were numbered in duplicate 1A, 1B, and 1C. At the writer's suggestion branch a of lateral 1 was opened 20 feet from its outlet and again at 735 feet from its outlet. The first of these two pits was underlain by marl. The second was in uncultivated peat and had no marl below it. Two samples were taken from each pit and numbered in duplicate 2A and 2B. The samples were set up in order on the running board of one of the cars and photographed by members of the party. One set was then chosen by the publicity director of the cement association and the other taken by the writer. The upper and lower sides of the samples taken by Elliott are shown in Plates 7, A and B. It will be noted that the tile which remained above the water table for long periods of time are very little attacked, while the tile which kept moist without much flow even though close to the marl is more disintegrated than those which carried a heavy flow of high ground water. Of the two from branch a, the tile taken from above the sand is more disintegrated than the one from above the marl. Samples of water were taken from the pits by the publicity man. On digging holes to the grade of the tile for the collection of water no water appeared. The holes were then deepened still more. At hole 1 B, the shovel handle was thrust down through the marl to the sand underneath. The water then rose in the hole. At pit 2 B, the hole was deepened almost to the underlying sand, permitting the water to collect. What was done with these samples is not known to the writer, but they can not be taken as representative of the water in the peat. They would be representative of the ground water in the sand subsoil, probably contaminated by the marl and peat.<sup>4</sup>

The samples of the tile taken by Elliott were tested on October 25 in the drainage laboratory of the experiment station. Crumbs were broken by hand from 1B, 2A, and 2B. All showed a strong alkaline reaction. The results of the physical tests are given in Table XVIII.

<sup>4</sup> Since the above was written the 1922 report of the American Society for Testing Materials has come to hand. It is believed that the samples tested by Dr. Witt and referred to in paragraph 3, p. 26: of that report, are the samples just mentioned.

TABLE XVIII.—Results of physical tests on samples <sup>1</sup> 2½ years in peat taken from drains at Coon Creek Aug. 1, 1921 (5-inch tile tested)

Physical tests.	Sample 1A.	Sample 1B.	Sample 1C.	Sample 2A.	Sample 2B.
Weight after 3 months drying (pounds)	9.70	9.02	9.19	9.27	9.53
Breaking load (pounds)	826	804	1007	810	1016
Absorption, bone dry and boiling method					
Top piece in mold	12.9	15.6	13.7	13.8	13.6
Center piece in mold	14.3	17.9	15.1	16.2	15.4
Bottom piece in mold	14.1	13.1	13.3	17.4	15.8
Average.	13.8	15.5	14.0	15.8	14.9

<sup>1</sup> A considerable number of tile had been left over when the job was finished, and had been stock piled on high ground at the edge of the marsh. Of these, 10 were taken as a sample, 5 being given to the soils department and 5 tested on October 25 in the drainage laboratory.

TABLE XIX.—Results of physical tests on concrete tile from stock pile at Coon Creek (5-inch tile tested, age 3 years)

Physical tests.	Sample 1-6.	Sample 1-7.	Sample 1-8.	Sample 1-9.	Sample 1-10.
Weight after 3 months drying (pounds)	10.27	9.30	10.25	9.61	10.00
Breaking load (pounds)	1152	983	1196	830	1265
Absorption:					
Top piece in mold	12.2	13.3	11.8	15.1	13.4
Center piece in mold	12.2	15.6	13.1	14.1	16.3
Bottom piece in mold	13.2	14.2	13.0	14.5	14.3
Average.	12.5	14.4	12.6	14.6	14.7

A comparison of the figures in these tests discloses some interesting facts that are tabulated in Table XX.

TABLE XX.—Comparison of physical tests on Coon Creek tile at different ages and under different conditions

Physical tests.	1 week after delivery.	3 weeks after delivery.	3 years old in stock pile.	2½ years old in peat.
Weight, air dry average	..	8.56	9.89	9.34
Wet weight	10.025	..	..	..
Breaking load, dry	..	910	..	..
Breaking load, wet	574	..	1085	893
Absorption, cold, per cent (estimated)	17.1	..	..	..
Absorption, boiling	..	..	13.8	14.8
Absorption, top piece in mold	..	..	13.2	13.9
Absorption, center piece in mold	..	..	14.3	15.8
Absorption, bottom piece in mold	..	..	13.8	14.7

TABLE XXI.—*Comparison of tile in peat with tile from stock pile at Coon Creek, tested Oct. 25, 1921*

Item.	Per cent.
Loss in weight . . . . .	5.5
Loss in strength . . . . .	17.8
Increase in porosity:	
Ends . . . . .	5.2
Middle . . . . .	10.4

Summary of Table XIX

## PEAT IN LAKE PRAIRIE DISTRICT

On September 19, 1921, a concrete tile line was examined on the NW.  $\frac{1}{4}$  and SW.  $\frac{1}{4}$ , sec. 29, township of Lake Prairie, about 10 miles northwest of St. Peter, Minn. In 1919, an 8-inch tile had been laid a short distance into a peat pocket about 1,200 feet long. The line was not completed. After eight months in the ground the tile was taken up and found to be very badly disintegrated on the bottom, as shown in Plate 6, B. New tile was laid in the fall of 1920 and the main completed. At the time of the examination it also showed signs of serious disintegration. No samples of the new tile were taken. Tests of the old tile showed physical conditions as summarized in Table XXII.

Table XXII.—*Absorption tests, bone-dry and boiling method, of concrete tile in peat 8 months, and on bank 1 year, near St. Peter, Minn.*

Sample taken.	No. 1.	No. 2.
Top piece in mold . . . . .	13.0	25.3
Center piece in mold . . . . .	16.6	19.3
Bottom piece in mold . . . . .	14.6	14.6
Average . . . . .	14.7	19.8

All of the fragments of tile No. 1 were from the upper side. The upper and center fragments of tile No. 2 were from the partially disintegrated portion. The tile were apparently of about the same character originally, a rather low grade without sufficient coarse aggregate.

## RESULTS

In addition to the 10 points brought out in the laboratory, the field studies indicate the following:

(11) Concrete tile, as at present made, break down in all peat soils, no matter what the underlying mineral soil may be.

(12) A high percentage of lime, even the presence of marl, is no guaranty of immunity.

(13) A high percentage of lime delays but does not stop the process of disintegration.

(14) An acid subsoil aids in the disintegration.

(15) The more porous the tile the more rapid the disintegration.

(16) The presence of water is necessary for disintegration to take place.

(17) It is not necessary for the tile to be actually in the peat for disintegration to take place, but merely that the peat waters shall have free access to it.

(18) The violent alkalinity of the tile, continuing even to the time of complete disintegration.

(19) The destruction of solid concrete if the acid waters lie against it continuously.

#### PART V. DISCUSSION AND CONCLUSIONS

The mechanical adaptability of such a material as concrete to the manufacture of drain tile and the aid which it can give to drainage work is very great. Its permanence in ordinary soils seems to be all that can be desired. That, however, is not a part of the discussion of this paper. It is with the suitability of concrete for peat soils or soils high in organic matter with which this paper deals.

From the facts brought out in the investigation it would seem that there are some things which should be remedied before concrete tile, as now made, can be said to be suitable for use in soils high in organic matter. The first of these difficulties is the presence of free alkali. The observations of the past year seem to indicate that this is a characteristic of all concrete tile, retained even to the time of complete collapse. If it is true that free alkalinity is an inseparable characteristic of concrete as now made, and if water is present as a conveying medium, then the ultimate destruction of the tile in the presence of organic acid seems inevitable. The free alkali and the free acid are incompatible and must react against one another. This might not be serious if the products of the reaction were insoluble in water. The investigation has shown that they are highly gelatinous and are very readily soluble in water carrying carbonic acid.

That carbonic acid is absorbed in large quantities by the concrete is indicated by the tests with the cement patties. It may also have had considerable to do with the increase in weight and density of the tile which lay in the stock pile at Coon Creek.

Destruction of the concrete by water carrying carbonic acid must eventually take place exactly as the lime is leached from the surface of any soil by the formation of the double carbonates of calcium, but this process probably would be extremely slow and would not be of economic interest to the engineer. It is the rate of decomposition that is the controlling factor.

#### DISINTEGRATION IN LOW-LIME PEATS

On this rate of decomposition, the character of the surrounding mineral soil, the porosity of the tile and the character of the ground water flow, seem to be the controlling factors. In strongly acid soils, with the peat wet throughout the season, ordinary tile appear to break down after one year. At Grand Rapids, Minn., the peat is highly acid but the tile were laid shallow and the ground water, except for short intervals, kept well below them through the greater part of the season. Some of the tile were in fair condition at the end of 11 years. At Weyauwega, Wis., in acid soil in the presence of abundant moisture, the tile were not placed directly in peat but in the sand below it. The system began to give trouble after two years and was very bad in three. In Wood and Juneau Counties, Wis., in acid peat above acid subsoil poor tile break down in a year.

## DISINTEGRATION IN HIGH-LIME PEATS

In the high-lime peats the situation is much more complex. Water still is a controlling factor. At St. Peter, Minn., a rather poor grade of tile broke down in a season. This was in a so called running slough in Prairie Country which would therefore be high in lime, but the bottoms of the tile were wet throughout the season. At Coon Creek, during the two and one-half seasons they were in the ground, the tile disintegrated most rapidly where there was a gentle flow or soakage of water regardless of the presence of lime in the form of marl. Where the flow of ground water was stronger, even though marl was not present, disintegration was not so great. Where the tile were well above the ground water level throughout the greater part of the season, disintegration amounted to very little.

On University Marsh the conditions were very unusual. The marsh surface was below the level of Lake Mendota. There is, in addition, heavy seepage from the surrounding hills. This seepage coming through the marl bed was so strong that the peat was prevented from attacking the bottoms of the tile. This is the reverse of what is commonly found. The tops of the tile were, however, above the seepage water and were destroyed after six years. This is the more remarkable in the light of the fact that during the winter the seepage was sufficiently strong to turn the deeper peat strongly alkaline and almost neutralize it to the surface.

In Ozaukee County the least proportional disintegration was found. The marsh in this case was narrow and the main ditch comparatively deep. The tile would be above the water level throughout the greater part of the season. As was to be expected, the greatest disintegration was found where the tile were kept continuously moist. In none of the three observations made in this county was there any serious disintegration after four years.

## PRODUCTION OF ORGANIC ACID

One of the most interesting facts brought out by the investigation was the extremely rapid production of organic acids where moist peat was kept at a warm temperature. In the laboratory culture of peats from University Marsh, both the peat from the surface, which was very faintly acid, and the peat from 5 feet deep, which was strongly alkaline, in 10 days became strongly acid. In another 10 days the surface peat contained 5 times as much acid as it did at the end of the first 10 days. The deep peat in the second 10 days not only increased its acidity to 7 times what it was at the end of the first 10 days but actually outstripped the shallow peat. The northern peat which was but faintly acid in the beginning became violently acid at the end of 10 days.

The same thing was done during this past winter with peat from Coon Creek. The peat culture in distilled water required a week to overcome its original alkalinity, after which time the increase in acid was rapid.

It is not assumed that exactly the same sort of accumulation of acid will occur in nature though the process of production may be identical. What will occur will be the production on an enormous scale of the products of decay. Much of these will naturally escape and be carried away by ground water. In the cooler months, particularly in the winter, the escape of the products of decay will probably more than keep pace with



their production. But in the summer months when run-off is light and decay rapid, the products of decay must accumulate to a very great degree. If there is any run-off through the tile lines, these products of decay will find their way to the tiles along with the drain water. The percentage of concentration of the acid in the drain water may be very low, but the actual amount passing during a season would be enormous. With the drain water also carrying carbonic acid, the complex compounds formed with the cement would be immediately carried away, presenting a fresh face to the action of the organic acids.

Though this process is evidently very similar to the leaching of lime from surface soil, a little thought will show that they are not exactly analogous. The lime in the soil is present in a comparatively inert form. It has been demonstrated that the concrete of the tile is chemically active even to the very end of the tile. It is possible that the ordinary soil is better able to retain the complex lime-organic compounds than is the peat. It has been shown that tile near the surface of a well-drained peat are not greatly affected. It is possible that the greatest injury is done by organic compounds that are produced in quantity only where the conditions are suitable. It is possible that these conditions may not exist in a mineral soil. This leads us to the statement that the deductions from this investigation can not be stretched to include the use of concrete tile in mineral soils whether acid or alkaline.

#### ECONOMIC FACTORS

Though, under certain conditions, the life of a concrete tile in peat soil may be very short, under certain other conditions it may continue a long time. In general, it may be said that the average life of concrete tile, as they have been made, is about six years. The tile may hold on for a considerable time during which the violent alkalinity is being neutralized, after which the final collapse is rapid. In peat soils the collapse of the tile may not be as serious as it would be in a mineral soil, nor may the collapse at once become apparent. If the drain is not closed by the tramping of stock or by farming operations, the underground channel may remain open for years, functioning nearly as well as the original drain.

It will probably be said that the quality and density of concrete tile is improving rapidly and that the tile at Coon Creek, for instance, were not good tile and were condemned before they went into the ground. Even though they were not good tile, however, they were no worse than many hundreds of miles of tile that have been used. They were shipped from the factory while still "green," and the first test was made when they were only partly cured. That is not the point, however. The point is that during the two years in the ground the tile lost in every quality that fits them for the work for which they are intended.

If the average expectation of the life of the tile were 40 or 50 years or upward, there would probably be no question as to the advisability of using concrete tile, but with an expectation of only 6 or 10 years it does not seem that, as now made, their use in peat is sound economics. It would seem to be a question whether or not they would pay for the investment and give a sufficient profit during that time to reimburse the land owners for the trouble and business hazard involved. No doubt improvements in the quality of the tile which are now being generally made will greatly increase the life of the tile, but those improvements are with few

exceptions leading to the improvement of one quality only, and that is density.

But density alone is not sufficient. In the progress of the field work, the greatest possible range of soils was investigated. It is significant that in no place where concrete tile were in peat for two or more years were the tile free from some percentage of disintegration. We can not assume that all of these tile, covering some 30 odd observations in two States, were below the average in quality.

#### IMPROVEMENT NECESSARY

It would seem that the first requisite for making the concrete permanent in a soil high in organic acids is the elimination of the free alkali. It is generally understood that an excess of lime is added to the materials of which cement is made in order to give it setting qualities and plasticity. If these qualities are necessary for a general purpose cement, it is possible that a special cement might be made for use in drain tile in peat soils which would not have the objectionable features of the standard product.

The second suggested improvement would probably be covered by the first, but if the first were not put into effect it would seem that a very distinct improvement could be effected by finer grinding. This would permit of more complete hydration at the time of setting. The "setting" of the old tile when it was reground would seem to indicate that perhaps the original particles of cement were too large for the process of hydration to be complete even after five years.

The third suggested improvement in the manufacture of concrete tile for use in peat soils is the addition of some substance which would either render the constituents of the cement chemically inert or would so coat the particles of the tile that the entrance of water would be prevented. If this could be done the tile would last as long as the coating remained intact. If such a material were used it must of necessity be of such a character that it itself would not decay.

The fourth method of improvement is the one that is at present being used. It involves greater care in the mechanics of manufacture of the tile, and includes washing, grading, and elimination of soft materials from the aggregate, better mixing and blending and better packing and curing. The result is a stronger, denser tile that is greatly superior to the tile made by a more careless process. Its life in any soil, but particularly in soils which carried inimical solutions, would be very greatly lengthened. It is possible that this method combined with the addition of a preservative substance will produce the desired results. Very great improvement has already been made along that line, and the utmost thought and care should be expended in an effort to produce a concrete that is unquestionably permanent.

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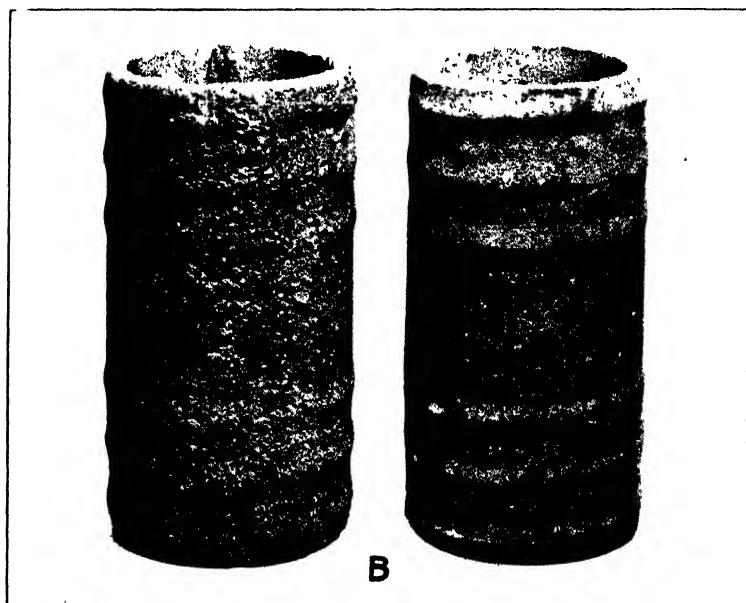
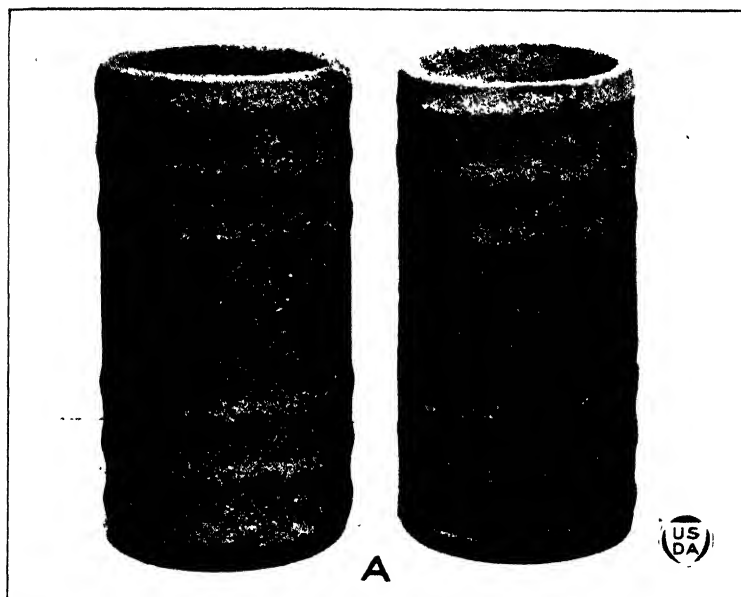
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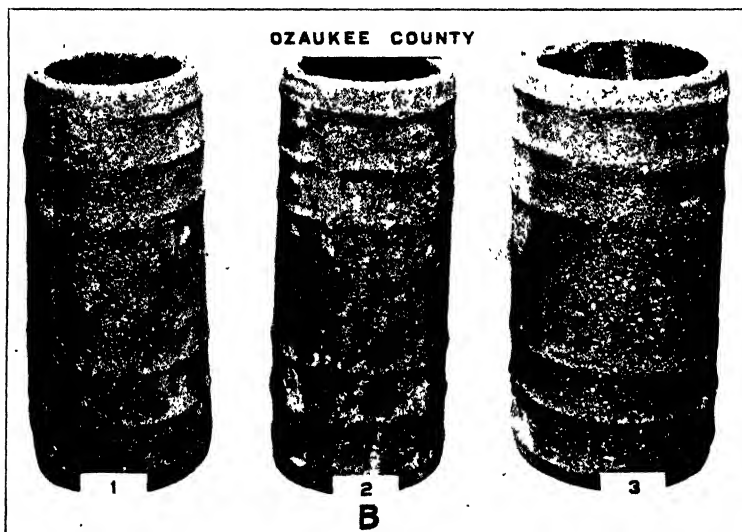
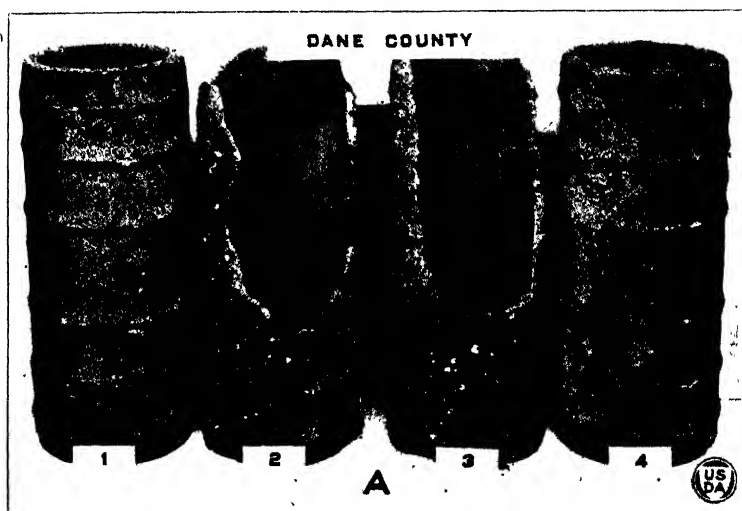


PLATE 1

A.—Concrete tile on left laid in deep peat on University Marsh in 1918. Taken up in 1919. The tile to the right is new.

B.—Concrete tile at left laid in peat  $3\frac{1}{2}$  feet deep underlain by marl in University Marsh in 1914. Taken up in 1919. The tile to the right is new.





## PLATE 2

A.—Concrete tile laid near Madison, Wis., in 1914, taken up in 1920. No. 1 has not been in the ground. Nos. 2 and 3 were laid  $3\frac{1}{2}$  feet deep in peat. No. 4 was in silt loam overlain by 2 feet of peat.

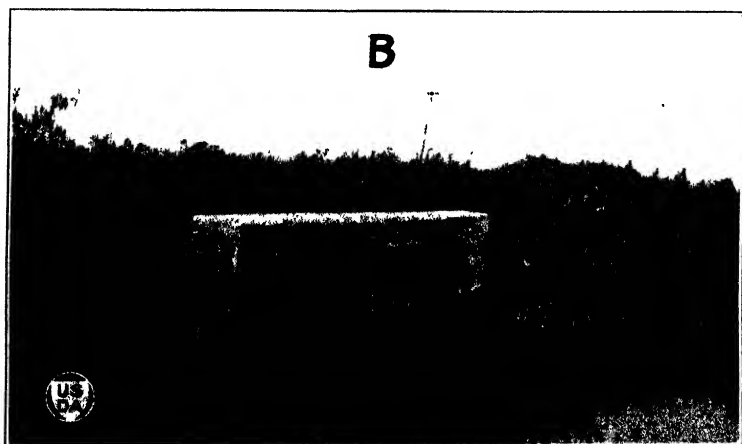
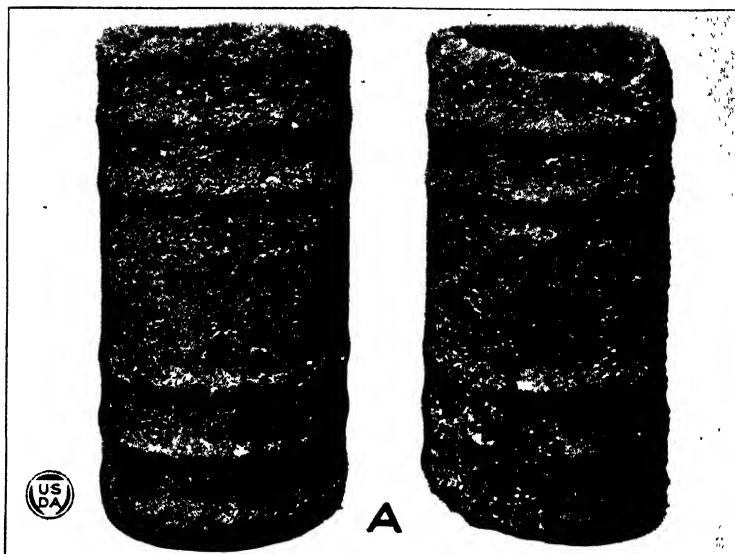
B.—Concrete tile laid in recent soil high in lime in 1916; taken up in 1920. No. 1 was in peat carrying a high percentage of alluvial matter and getting high ground wash. No. 2 was at the outlet of a drain in 4 feet of peat. No. 3 was in  $4\frac{1}{2}$  feet of peat overlying sand.

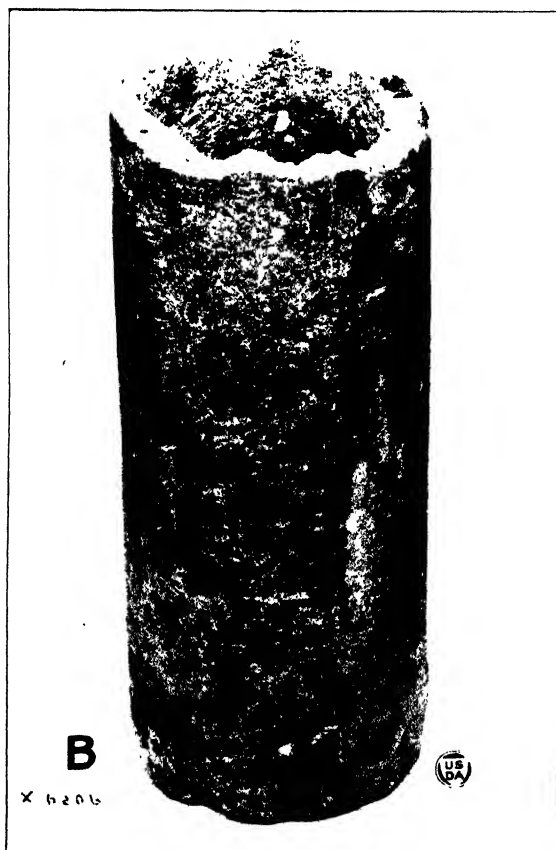
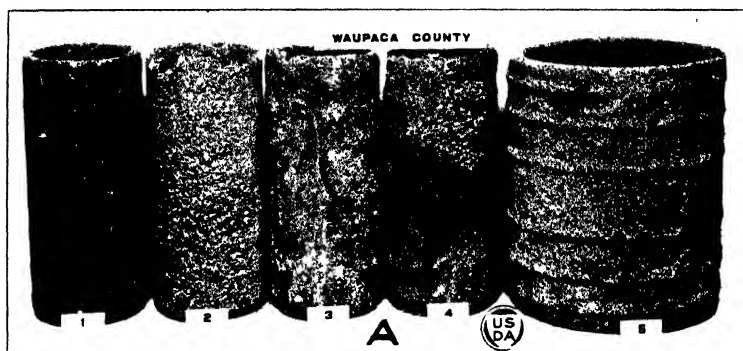


### PLATE 3

A.—Tile from University Marsh. From same line as in Plate 1B, but taken up in fall of 1920. Note progressive disintegration in Plates 1, A, 1, B, and 3, A.

B.—Concrete culvert in Gaynor Road, Grand Rapids, Wis. Built about 1910. Photographed September, 1920. Acid water from the cranberry marshes stands against the concrete at all times.





#### PLATE 4

A.—Concrete tile laid in Central Plain of Wisconsin in 1915, taken up in 1919. No. 1 lay on the ground. No. 2 was in sand below 8 inches of black muck. No. 3 was in silt loam with sand at 3 feet 6 inches. No. 4 was in bowlder clay underlying 12 inches of black muck. No. 5 was in sand below 8 inches of black muck.

B.—Concrete tile laid in a sewer in Milwaukee about 1885. Taken up in 1919

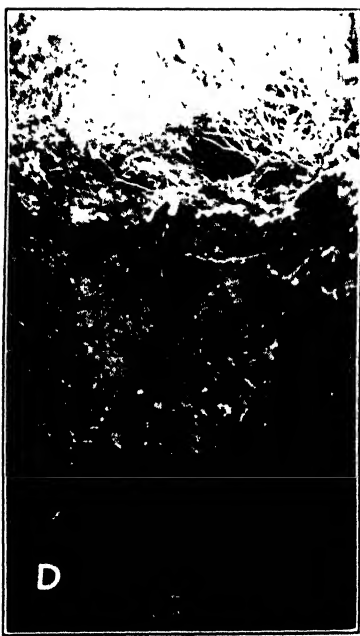
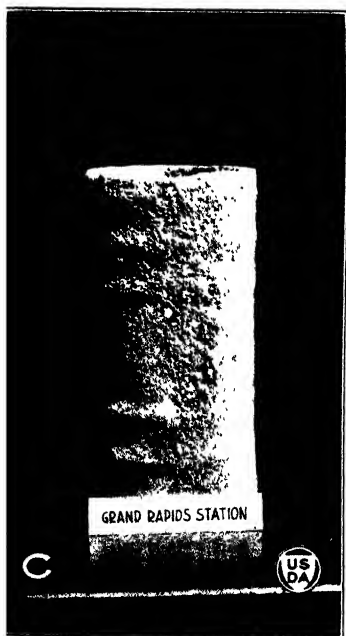
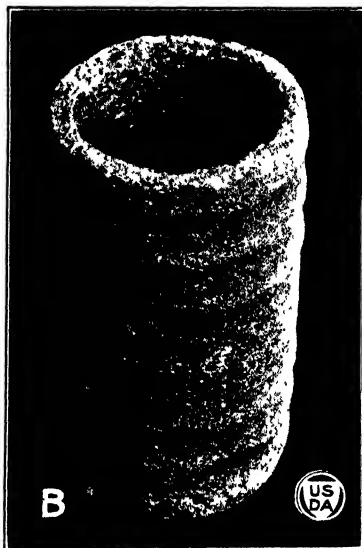
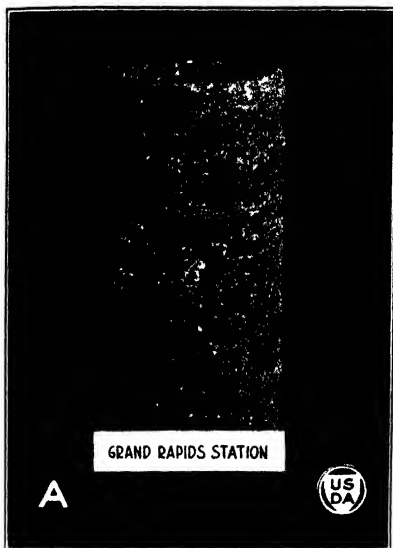
PLATE 5

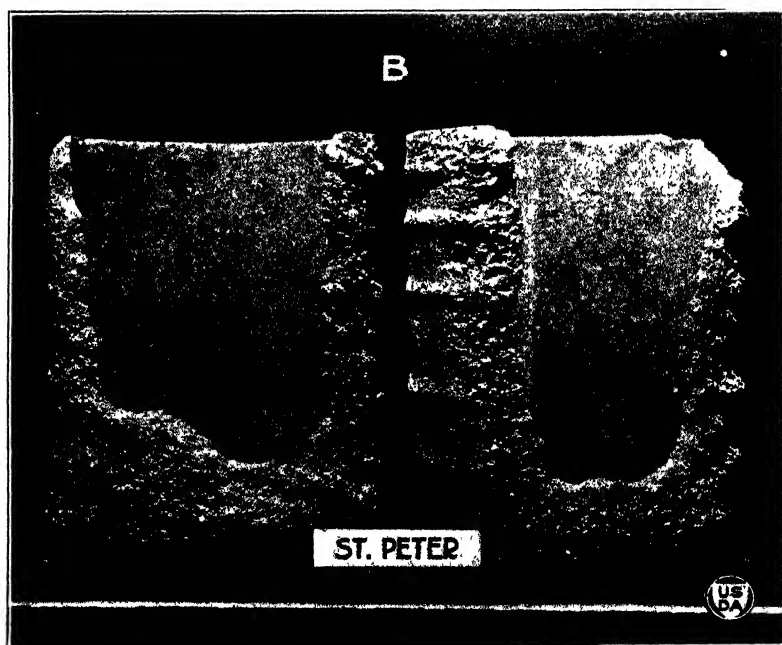
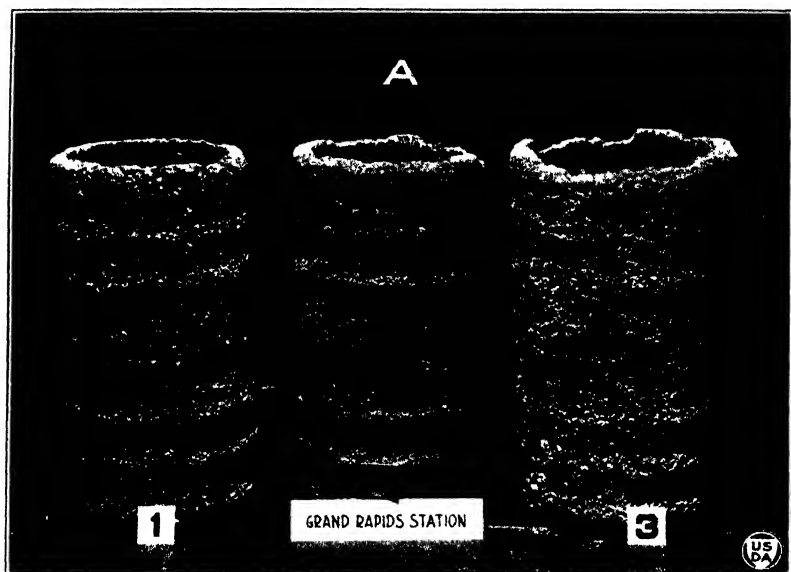
A.—Tile from Grand Rapids Experimental Farm. Laid 1910 Taken up 1918.  
Laid in pile until 1921.

B.—Same as Plate 5, A, showing tile disintegrated on inside at bottom.

C.—Similar to preceding, showing disintegration at water line.

D.—Grand Rapids station, showing sample No. 2 in the ground with the peat arched above it.





#### PLATE 6

A.—Some of same lot laid 1910. Taken up 1918. About one-quarter of tile discarded; remainder relaid. Samples taken up 1921. No. 1, bottom of tile in deep peat. No. 2, top of tile in deep peat. No. 3 taken from  $3\frac{1}{2}$  feet deep in mineral soil. Seepage line, but carried peat waters. This portion of line had not been relaid since 1910.

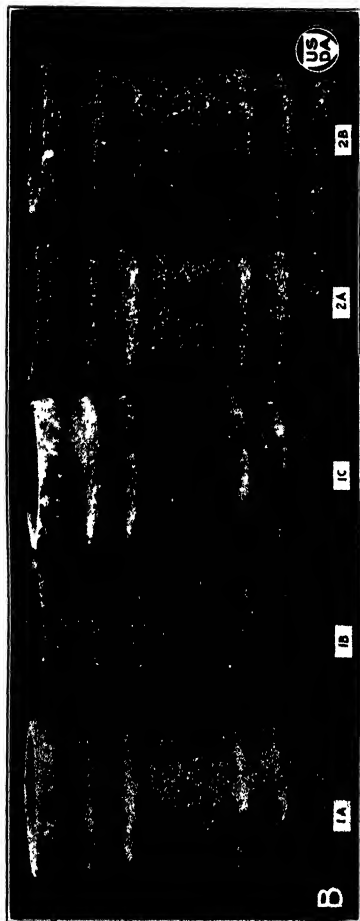
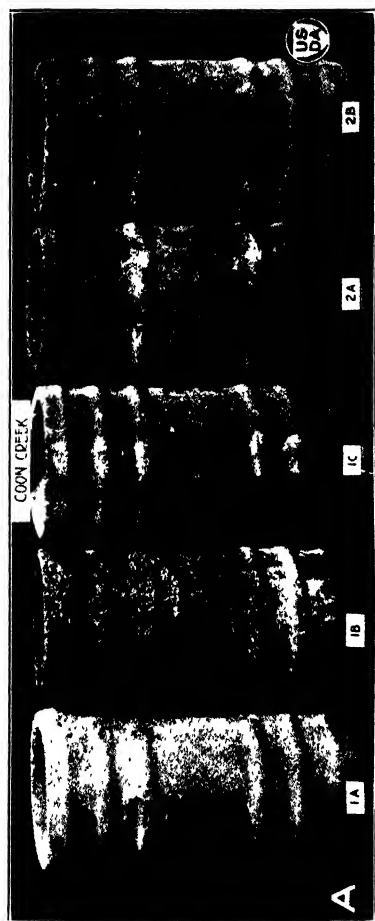
B.—Eight-inch tile from line in peat northwest of St. Peter. Laid in 1919. Taken up after eight months. Second set, relaid in 1920, showed signs of heavy disintegration in September, 1921.



# PLATE 7

A —Upper side of set of tile from Coon Creek, taken by Elliott for testing, 1A, 1B, and 1C taken from pits on lateral 2, peat underlain by marl; 2A and 2B from pits on branch a of lateral 1, 2A peat underlain by marl, 2B peat underlain by sand

B.—Bottoms of same set as shown in A.





# INJURY TO FOLIAGE BY ARSENICAL SPRAY MIXTURES<sup>1</sup>

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## INTRODUCTION

Since the first trials of Paris green and of arsenic trioxid for the control of the potato beetle half a century ago there has been a constant increase in the use of arsenical compounds for controlling the ravages of certain species of insects on economic plants.

It is a notable fact that of the many poisonous substances known to physiological chemistry none has been found so well suited to the purpose of poisoning insects that obtain their food by gnawing away portions of the fruit and foliage as certain arsenical compounds. As the control of such insects is a very important field in the science of entomology, much practical and scientific interest centers around this group of compounds. This interest makes imperative the obtaining of more information along certain lines; for it is agreed that an arsenical compound to be a satisfactory insecticide for application to foliage shall approach perfection in these respects: (1) It must promptly kill a large proportion of the insects; (2) it must be relatively inexpensive; (3) it must not seriously injure the plants to which it is applied under the conditions obtaining. Unfortunately, no compound has yet come to notice that perfectly satisfies these requirements, though several do so sufficiently well to be extensively used. It is with the third requirement that this investigation deals, although the others are constantly kept in mind.

The problem of arsenical injury to fruit trees and garden crops as a result of spraying is a troublesome one. Some of the compounds first tried have been abandoned or greatly restricted because of the injury produced and new ones have been proposed to take their places; and these in turn may yet give way to others.

It has long been evident that there are factors influencing arsenical injury that the horticulturist does not understand and others that he is powerless to control. Considerable work has been done to show the nature and relative importance of these factors. All of this work has been fragmentary and most of it has been done under such conditions that it is impossible to judge the relative importance of two or more factors operating at the same time. In most cases the exact composition of the mixture used was not known and in many cases the fact that quite different chemicals appear under the same name evidently was not even suspected. Some of the conclusions drawn are quite contradictory, and others, though perhaps correct, are based on so little evidence that their soundness is questioned. It is probably safe to say that at the time this investigation was undertaken at this station in 1912 enough correct conclusions on this subject had already been drawn to make certain phases of this work largely unnecessary if these correct conclusions had been recognized and isolated from the mass of apparently conflicting data and theories. Such a distinction had, however, been found impossible.

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<sup>1</sup> Accepted for publication Sept. 2, 1922.

## ARSENICAL COMPOUNDS USED AS INSECTICIDES

As some of the confusion on the subject of arsenical injury can be explained by a study of the processes of manufacture resulting in somewhat different compounds being sold under the same name, a brief review of these processes will be in order. It will also be necessary to discuss briefly the use of the more important arsenical insecticides that the significance of the work that follows may be evident.

## ARSENIC TRIOXID

## PREPARATION

Arsenic trioxid is obtained in large quantities as a by-product in roasting arsenical ores. This is not pure, but contains some metallic arsenic, arsenic sulphid, dirt, etc., from which it is purified by resublimation. There are two forms of the trioxid known, the amorphous and the crystalline. The amorphous changes slowly into the crystalline form under some conditions. At 25° C. the amorphous form is soluble in about 30 parts of cold water, while the crystalline requires about 100 parts. Both are slowly but completely soluble in about 15 parts of boiling water. The above proportions are approximate, as there seems to be a lack of agreement in the statements of various writers in regard to the solubility of the oxid. The name "arsenious acid," by which it is often referred, is a misnomer, since the oxid is an acid anhydride and has no acid properties until it unites with water, when arsenious acid is formed. In all probability the caustic effect of arsenic trioxid on vegetation is due to its combining with water and forming arsenious acid. Therefore the injury is proportional to the amount of arsenious acid formed and not to the amount of arsenic oxid in suspension.

## USE AS AN INSECTICIDE

According to Bourcart (*1*, p. 95)<sup>2</sup> the first trials of using arsenic trioxid for spraying were conducted in America in 1867, when Markham used it to combat the Colorado potato beetle. On account of the serious injury occurring when it was used, other less soluble arsenical compounds were substituted until at the present time the oxid is used almost exclusively in the preparation of poison baits, and as a constituent in the preparation of some of the more usable arsenical compounds.

## CALCIUM ARSENITE

Arsenite of lime was probably first recommended as an insecticide by Kilgore (*14*). He recommended that it be made by boiling together for one-half hour, in 2 to 5 gallons of water, white arsenic 1 pound, lime 2 pounds, and diluting the required volume to, say, 100 gallons. It is desirable that the lime should be present in the boiling solution of white arsenic, since it renders the latter insoluble as fast as it goes into solution, thus reducing the volume of water and shortening the time for obtaining the arsenite. Calcium arsenite is not manufactured for an insecticide, but many cases of foliage injury have been reported from its use, showing that it is more or less dangerous to use, and for this reason is not generally recommended.

<sup>2</sup>Reference is made by number (*italic*) to "Literature cited," pp. 535-537.

## COPPER ACETO-ARSENITE (PARIS GREEN)

## PREPARATION

Copper aceto-arsenite is a compound of copper, arsenic, and acetic acid,  $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot \text{Cu}_3\text{As}_2\text{O}_8$ , and theoretically contains 58.65 per cent arsenious oxid ( $\text{As}_2\text{O}_3$ ), 31.29 per cent copper oxid ( $\text{CuO}$ ), and 10.06 per cent acetic acid,  $\text{C}_2\text{H}_3\text{O}_2$ . In the process of manufacture verdigris and arsenic trioxid are the essential materials. Generally verdigris is dissolved in acetic acid and added to a boiling solution of white arsenic and allowed to stand for some time to completely precipitate the copper aceto-arsenite. The light green precipitate is then thoroughly washed with hot water to remove the soluble salts.

Copper aceto-arsenite has several synonyms, the most important of which are Paris green, Schweinfurt green, and emerald green. The compound was discovered in 1814 during the course of experiments, with the object of preparing an improved Scheele's green or arsenite of copper for use as a coloring pigment in the arts. It is supposed to have been first made at Schweinfurt, Germany, but by whom is not recorded. On account of its poisonous nature its use as a pigment was limited, and at present it is scarcely used at all for this purpose, but has become one of the standard insecticide compounds, not only in this country but abroad.

## USE AS AN INSECTICIDE

The history of the use of Paris green as an insecticide may be traced back to the period when the Colorado potato beetle became recognized as being of economic importance. The first published account of the destructive propensities of this beetle may be found in the *Prairie Farmer*, August 29, 1861, (7) and Paris green appeared upon the scene at some time between 1860 and 1870, but who first suggested it and who first used it for the destruction of the potato beetle will perhaps never be told. The use of this material as a standard insecticide undoubtedly began in the West Central States. At first the poison was applied in the powdered form, using flour, plaster, or ashes as a diluent. In 1872 Le Baron (15, p. 116), State entomologist of Illinois, in referring to the spring cankerworm, recommended that—

strong washes, such as Paris-green water, or suds made from whale-oil soap, thrown upon the trees with a garden syringe, will also materially check their depredations. This is probably the first statement in which the syringing or spraying of apple trees with Paris green is recommended. The first statement referring to the successful control of codling moth by the use of Paris green that attracted attention and which was followed by close investigation, appears to have been made by Edward P. Haynes (Lodeman, 16, 1910) in 1878. After spraying his orchard with Paris green he said that it not only rid the orchard of cankerworms but the apples on the sprayed part were much less eaten by codling moths. However, the use of this poison for the destruction of foliage-eating insects was for various reasons adopted slowly, and it was not until about 10 years later that Paris green was freely recommended as one of the most valuable insecticides for the destruction of chewing insects.

## FERROUS ARSENATE

The use of arsenate of iron as an insecticide in this country is in an experimental stage and this compound has been used to only a limited extent.

## PREPARATION

In France, Vermorel and Dantony (32), who experimented with iron arsenate for several years, recommended that it should be prepared as follows in order to get the maximum adhesive power: 400 grams ferrous sulphate in 10 liters of water is added to an equal amount of arsenate of soda in 10 liters and stirred constantly. This stock solution is diluted to 100 liters and will contain about 200 gm. ferrous arsenate per hectoliter.

## USE AS AN INSECTICIDE

Upon its use as an insecticide Smith (28) reports the use of arsenate of iron containing 45 per cent arsenious oxide on elm at the rate of  $1\frac{1}{4}$  pounds to 100 gallons of water without injury, and that it had good insecticidal qualities. Vermorel and Dantony (32) used iron arsenate against the codling moth in 1906, 1907, and 1908 and concluded that the action of iron arsenate is equal or superior to lead arsenate, with the advantages of color, presence of iron versus lead, and cheapness. They concluded that the combination of iron arsenate with copper fungicides is not practicable, first, because such combination is not necessary, and, second, because it is very destructive to foliage, and recommend that iron arsenate be used alone and in no case combined with copper. Taylor (31, p. 15) reports the use of iron arsenate against the fall webworm in 1909, using 1 to 3 pounds to 50 gallons with promising results and no injury to peach foliage. Melander (19) says iron arsenate is a new spray that has the merit of cheapness, but that it is not on the market. He gives brief directions for its preparation, but does not discuss results obtained from its use. Scott and Siegler (25) concluded that iron arsenate was a slow-acting poison. In field tests on apple they found that it was not an effective insecticide for the codling moth used at the rate of 1 pound to 100 gallons. When used at greater strengths, however, they thought that it should give fairly satisfactory results, but that it would have no advantage over arsenate of lead.

## LEAD ARSENATES

## PREPARATION

Arsenate of lead for use as an insecticide is usually prepared by one of two methods, according to Haywood and McDonell (12). First, by using lead acetate and disodium arsenate when a precipitate of triplumbic lead arsenate,  $Pb_3(AsO_4)_2$ , is formed, or, second, by using nitrate of lead and disodium arsenate when a precipitate of diplumbic lead arsenate ( $PbHAsO_4$ ), is formed.

## COMPOSITION

Theoretically the tri-plumbic lead arsenate contains 74.40 per cent of lead oxid ( $PbO$ ), 25.60 per cent of arsenic oxid ( $As_2O_5$ ), and 2.59 per cent water of constitution.

On account of various conditions, the commercial lead arsenate only approximates the percentages given above. This will be obvious for the following reasons: Chemically pure salts are too expensive to use in its manufacture, while various conditions such as temperature, concentration, etc., affect the reaction indicated by theory. Recent work by some investigators tends to show that the lead arsenate commonly on

the market is not either of the above forms, but is a double salt of a complex nature. For the purpose of this paper the lead arsenate will be designated as one of the above forms, or a mixture containing the two forms.

#### USE AS AN INSECTICIDE

Arsenate of lead was first used as an insecticide during the summer of 1893. In the early work of eradication of the gipsy moth it became evident that the known arsenicals could not be used at the requisite strength for killing gipsy moth caterpillars without serious injury to the foliage. Arsenate of soda was suggested as a substitute, but when used it burned the foliage to a greater extent than the other arsenicals. Mr. F. C. Moulton, who was experimenting upon these insecticides during the winter of 1892-93, proposed acetate of lead to precipitate arsenate of soda and in this way obviate the burning of the foliage caused by the latter; and by this method the first arsenate of lead was produced for insecticidal purposes. Arsenate of lead has the following advantages: (1) On hardy foliage it can be used at almost any desired strength without serious injury, (2) it is visible whenever used, and (3) it adheres well. The principal objection is its rather slow killing properties, probably due to its comparatively low arsenic content and slow solubility.

#### INJURY TO FOLIAGE

When lead arsenate was first used it was thought to possess all the necessary qualifications for an ideal insecticide. It has proved of great value and is used very extensively on apple and other more hardy foliage, but reports from its use on peach are variable, sometimes no injury being reported and in other cases the injury being so severe as to defoliate the trees. Fernald (8, 9) states that—

it [arsenate of lead] can be used in large proportions, if necessary, even up to 25 pounds to 150 gallons of water, without injury to the foliage. . . . It does not injure the foliage of the most delicate plants, even when used in as large a proportion as 25 pounds, or even more, to 150 gallons of water.

Smith (26, p. 437, 27, p. 8) states:

Its great advantage is its harmlessness to plant life of all kinds . . . it is absolutely harmless to foliage at any strength . . . It is the only effective poison of this character that can be safely applied to peach foliage and on conifers.

Many other statements similar to the foregoing are available to show that many considered lead arsenate as almost an ideal insecticide. However, other investigators conducting careful experiments occasionally reported serious injury. Haywood and McDonnell (12) say:

Rather severe injury was caused to the foliage and fruit of the peach by pure lead arsenate, made either from lead acetate or lead nitrate.

Woodworth (36) states, in speaking of spraying in the Pajaro Valley with lead arsenate, that:

While the codling moth was well controlled, the amount of burning was so large that the progress of spraying was entirely checked. . . . The most significant discovery of the year 1906 was that where a lead arsenate was so compounded that all the arsenic acid present was combined with lead no injury was produced on the most delicate foliage. Such a compound is known as the neutral or ortho-arsenate of lead. At that time no manufacturer was able or willing to produce an arsenate of lead of this description, and to this day, excepting the product manufactured here at Watsonville, there is no strictly neutral lead arsenate on the market.

These statements serve as illustrations that foliage injury is not due directly and entirely to the arsenical but is greatly influenced by other



conditions. Haywood and McDonnell (12) point out the great variation in the composition of the different samples analyzed, and this, no doubt, has had very much influence on contradictory results. Lead arsenates which are safe to use under dry arid conditions may cause serious injury under conditions of high humidity. Atmospheric conditions following sprayings have a great influence on the action of the spray mixture of the foliage, and for this reason duplicate experiments, when a short time intervenes between them, may not control. Gillette (11), Woodworth and Colby (37), and others concluded that leaves kept perfectly dry can hardly be injured by the arsenites, but that under conditions of high humidity the injurious action is greatly increased. However, at the present time lead arsenate is recognized as the standard insecticide, not only for orchard spraying but for field and truck crops as well. Against insects it is known as a rather slow poison, but it is effective. In this respect there is considerable difference in the two forms, viz, the triplumbic and the diplumbic ortho-lead arsenate, due in all probabilities to the difference in their arsenic content, which is the poisonous principle.

#### LONDON PURPLE

##### PREPARATION

London purple consists of calcium arsenite, calcium arsenate, and inert ingredients as dye residue, dirt, etc., and is prepared by boiling a purple residue from the dye industry with slaked lime. The analysis on a package recently received at this station was as follows: Total arsenic (As), 21 per cent; active ingredients, calcium arsenate and calcium arsenite, 54 per cent; inert ingredients, dyestuffs, etc., 46 per cent; arsenic (As) in water soluble forms,  $7\frac{1}{2}$  per cent. Analysis by our station chemist substantiated the claim as to the total arsenic content. If judged from the arsenic content, London purple has only about one-half the killing strength of Paris green when used as an insecticide.

##### USE AS AN INSECTICIDE

The name "London purple" was suggested by Dr. C. E. Bessey (private correspondence) in 1878, and he was the first to use it as a substitute for Paris green for the destruction of the potato beetle. Doctor Bessey was one of the three men who first received sample packets of London purple sent by a London firm<sup>3</sup> in a letter dated September 7, 1877.

On October 2, after an exchange of letters, the firm forwarded to him three kegs of the material, which was the first shipment sent to America. The results of the experimental work of Bessey and Budd were favorable to the new poison, and it was soon recommended as a substitute for Paris green, not only for the destruction of the potato beetle but for other insects as well.

The value of this material was recognized with surprising rapidity, probably due to its cheapness and the ease with which it could be applied. The principal objection to its use was its injurious action on the foliage of plants. Its burning tendencies were undoubtedly due to the soluble arsenic it contained, and it was not uniform in composition. The early analysis of London purple shows variations in amounts of arsenious oxid of from 31 per cent to nearly 60 per cent. However, at the present time the manufacturers claim to have perfected a process by which the composition of London purple is as uniform as that of Paris green or others of the standard insecticides.

<sup>3</sup> Hemingway & Co.

From about three years after its introduction, London purple was generally considered to be nearly equal in efficiency to Paris green, and it is so considered to-day.

#### ZINC ARSENITE

In 1903, an investigation of arsenicals and spray injury was begun by the California Experiment Station. In the course of this investigation, foliage tests were made with zinc arsenite in the summer of 1906. In 1907, about 5 acres of apple trees were sprayed during the blooming season without injury, and the results indicated that this material was a promising insecticide. In 1909, the first commercial material was prepared by the California Spray Chemical Company, Watsonville, Calif.

#### PREPARATION

The process of manufacture in general consists in boiling together in water, in the presence of ammonia, zinc oxid and arsenious oxid, in approximately the proportion of their combining weights. The boiling is continued until the oxids combine, which is indicated by a marked thickening of the mass, and until the filtrate shows only traces of arsenious oxid.

#### USE AS AN INSECTICIDE

At the present time zinc arsenite is used extensively in certain localities for the spraying of apple trees just after full bloom, at the rate of 3 pounds of the powder to 100 gallons of water. It is not used for later spraying on account of its tendency to injure foliage; but from recent tests it is possible that it will become an important insecticide for truck crops.

#### INJURY TO FOLIAGE

Luther (letter of May 20, 1910) advocated the use of zinc arsenite on apple, pear, bean, and potato, but not on delicate foliage like peach. Volck (33) pointed out what he believed to be a fallacy in accepting the foliage of any one plant as a reliable index to the injurious action of all arsenicals. He found that either bean or peach foliage was a suitable indicator for testing arsenate of lead that is intended for use on apples. Because of the great ease of obtaining bean foliage, it was adopted as a standard testing medium. Later he says:

Arsenite of zinc may prove entirely neutral to bean foliage and yet when applied to peaches do marked injury. Samples which injure peach will later prove injurious to apples if applied in sufficient quantities, and bean foliage is not suitable for testing samples

Luther (17) in speaking of investigations in the Pajaro Valley states:

On apples it [zinc arsenite] has been sprayed as thick as whitewash without the least bit of injury. On small field crops, such as beans, potatoes, etc., it has given no injury, but on the peach, which is supposed to be more hardy than the bean, the injury was severe.

Woodworth (36), in speaking of spraying conditions in the Pajaro Valley, says:

This [zinc arsenite] has proven to be the safest of the arsenicals that can be procured in the form of dry powder. It is not so safe, of course, as the neutral lead arsenates, but has been used without very serious evidence of burning in the orchards where dusting has been adopted instead of spraying. . . . There is no doubt that the zinc arsenite stands foremost at the present time among the available arsenicals with high arsenic content.

Cooley (3) reports its use on the foliage of potato and cabbage without injury. Johnston (13) reports the use of zinc arsenite in the proportions of 1, 1½, and 2 pounds to 50 gallons of water on potatoes, without foliage injury. Clinton and Britton (2) report two applications of zinc arsenite on apple trees at intervals of seven days, using ¾ pound to 50 gallons, with foliage injury so severe that the trees dropped many of their leaves. Volck (34) recommends the substitution of zinc arsenite for lead arsenate for the first two sprayings, i. e., full bloom and 10 days later, but in the following combination: Zinc arsenite (dry basis), 6 pounds; iron sulphid, 6 pounds; Black leaf 40, 1 pound; water, 200 gallons; because when iron sulphid is added, the foliage-injuring properties are largely restrained. If used alone, only the first (full bloom) spraying should be applied. For later sprayings arsenate of lead is recommended.

Melander (19) says "It [zinc arsenite] is easy to use, adhesive, and has not scorched in our tests [on apple]." Schoene (24) found that on apples zinc arsenite alone or in combination with soap, glucose, or lime sulphur caused more or less injury, but that lime or Bordeaux prevented this injury. In other experiments with zinc arsenite, slight injury occurred on the foliage of pear and plums, peach and grape leaves were severely scorched, while potatoes and cabbage were uninjured. It is also suggested in this bulletin that the solvent action of carbonic acid is partly responsible for the damage. Scott and Siegler (25) record injury to foliage of apple when zinc arsenite was used alone at the rate of ¾ pound to 50 gallons of water or combined with milk of lime or with lime sulphur; that it caused moderate burning on bean foliage, except where lime was added, in which case no burning resulted; that when added to slaking lime for Bordeaux mixture it caused no foliage injury to either apple or grape; and they suggested its possible use with Bordeaux mixture in certain sections for the control of codling moth, bitter rot, and blotch.

By various workers zinc arsenite is considered an effective insecticide against insects that are rather resistant to the poisoning effect of arsenic, such as larvæ of webworm, tussock moth, etc.

#### METHODS OF INVESTIGATION

It has been apparent for some time that there are several factors that influence the injury of foliage by arsenical compounds. Some of these are inherent in the plants themselves, some are dependent upon the chemical nature and solubility of the compounds used, while still others are to be classed as environmental conditions. It is obvious that to determine the relative importance of these several factors one of them must be varied while the others are kept as nearly constant as possible, for it is quite impossible to eliminate all but one.

#### METHODS OF APPLYING CHEMICALS

A large part of the spraying was done in the college orchard at Bozeman, Mont.; but this was quite extensively supplemented by orchard work in other parts of the State, and by spraying plants of various kinds in field plots, on the college campus, and in the botanical greenhouse.

The mixtures were applied with a bucket spray pump under good pressure, using a Bordeaux nozzle. In the case of small plants, such as potatoes, sugar beets, etc., entire plants were sprayed, but on orchard trees this was rendered impossible by the very large number of applications.

that were made, reaching into thousands. In orchard spraying, therefore, a single branch 2 or 3 feet long was sprayed with each mixture, and it is probable that as accurate results were secured as though entire trees had been covered. Indeed, it was possible to cover these single limbs more evenly than whole trees could have been covered.

It has been found most convenient to use two liters of spraying mixture for each application, and for this reason the strength of the chemical used is indicated as the number of grams in two liters. This method of expression will be found in most of the tables in this paper.

#### PRECAUTIONS

Care was taken to avoid spraying on windy days, and if rain followed the application the results were rejected excepting as they could be used for data in relation to precipitation. For the regular work foliage was chosen that was normal in development and free from mechanical or other injuries. Both the upper and the under side of every leaf was drenched thoroughly, and except in special cases care was taken not to shake off the spray mixture before it dried upon the leaves.

As the number of applications exceeded 6,000 in the orchard and plots and 4,000 in the greenhouse, it is evident that the greatest care was necessary to prevent errors in labeling, recording, etc., if the results were to be thoroughly reliable. This was realized from the first, and a complete and yet simple system of checking was adopted and scrupulously followed. From the nature of this system and from the fact that in the checking an error in the original record was very rarely found, we feel assured that the few erratic results that appeared were all due to other causes.

In presenting these data comparisons are not made in the same table between tests run under different conditions, and the reader is warned not to make direct comparisons between injuries which resulted from spraying on different dates, in different places, or under other differing conditions, except for the purpose of studying the effects of these specific conditions, others being practically constant.

#### DEVELOPMENT AND CHARACTER OF INJURY

When a leaf is materially injured by arsenic, it shows visible symptoms in a day or two which become more and more pronounced until certain portions or, in severe cases, the whole leaf is dead, brown, and more or less shriveled. In severe cases the leaves drop in from one to four weeks from the time of treatment. Most commonly the first visible symptoms appear on the second day after treatment. At this stage there is very little change in color, but the surface of the leaf in the injured portion has lost its normal luster and becomes duller in appearance. The tissues under these duller areas have lost much of their turgidity and become more or less flabby. Very soon, perhaps the second day, a dull brown tinge is apparent, which at first is indefinite in outline and becomes more and more sharply defined. After 10 days of treatment no further change takes place except that the dead portion becomes frayed by the whipping of the leaves in the wind, or, if it is severely injured, the leaf drops off. No exact time can be given for this course of development, as it is hastened by hot, dry weather and retarded by cool, wet weather. Most commonly the first visible injury may be detected on the first or the second day. The condition shown in Plate 1, A may be seen on the

third day; Plate 1, B on the fourth day; Plate 1, C on the fifth day; Plate 1, D at the end of a week; Plate 1, E in about 10 days, and Plate 1, F in 3 or 4 weeks.

If the leaf is not entirely killed, as was usually the case in these experiments, the injury is worse at the margin and in roundish spots of all sizes in the interior. If there are abrasions through the epidermis, these become centers of discolored areas. In most of these spots, however, there is no visible mechanical injury, nor do they correspond to depressions in the leaf where the spray mixture collected in greater abundance.

The degree of injury was measured by the proportions of injured to uninjured surface. In the case of the apple, if more than half of the leaf is killed it usually drops prematurely. The position of the injured area varies this rule somewhat. Injury near the midrib or near or on the petiole is more likely to cause dropping than if on the margin, especially toward the tip. Not all plants are equally inclined to shed their leaves. The bean, for example, will do so much more readily than the apple, while the tomato retains them more tenaciously.

For the purpose of comparing results for tabular data, etc., a condensed method of expressing the degree of injury is highly desirable. For this purpose the terms, very slight, slight, moderate, bad, very bad, partly defoliated, and defoliated were adopted. As applied to individual leaves the following definitions apply to the records in this paper:

Very slight=the least amount of injury that is easily seen.

Slight=a few small spots up to one-eighth of the area of the leaf killed.

Moderate=one-eighth to one-fourth of the area killed.

Bad=one-fourth to one-third of the area killed.

Very bad=one-third to nearly all of the area killed.

As applied to the sprayed plants or branches as a whole the following definitions served as a guide:

Very slight=a few small spots on a small proportion of the leaves; no leaf seriously injured.

Slight=about one-eighth to one-fourth of the leaves showing spots, but few, if any, of them seriously injured.

Moderate=about one-third of the leaves more or less injured. Most of the leaves showing only small spots, but a few of them may be injured to the degree classed as "bad." In the definitions given above for individual leaves we have applied this term to the most serious injury that can be done without commercial injury to most crops.

Bad=approximately half the leaves injured, most of them only slightly, but some in "moderate" degree. This we have considered to be just enough injury to class as of commercial importance to most crops.

Very bad=most of the leaves more or less injured, some of them nearly killed.

Partly defoliated=a portion, but not all of the leaves entirely killed or so badly injured that they dropped off before the notes were taken. The leaves of some kinds of plants when all or nearly all the tissue is killed, will drop off. The leaves of other plants, as tomato, still cling after death, but for uniformity we class as defoliation a killing of the leaves whether they actually drop off or not.

Defoliated=practically all the leaves are killed.

Occasionally there would be some combination of injured leaves other than those listed above, as, for example, a few leaves badly injured and the others practically all sound, and judgment had to be used in designating the degree in such cases, but these definitions will, in general, serve to place the authors and readers on common ground.

#### CONDITIONS AFFECTING THE AMOUNT OF INJURY

It is a well-recognized fact that arsenic and its compounds tend to injure all forms of life with which they come in contact, but the degree of injury varies with several conditions, some of which are more or less under the control of the entomologist, the horticulturist, or the farmer, who makes use of these compounds for destroying insects upon growing crops. A study of these conditions constitutes the most important part of this investigation.

#### DIFFERENCE IN SUSCEPTIBILITY OF PLANTS

It has been found that different species of plants vary greatly in their natural resistance to arsenical action. Confining ourselves to the higher plants and especially to those that for economic reasons are likely to receive applications of arsenical insecticides, we find that some will be almost or quite killed by applications that will injure others but little.

#### DIFFERENCE IN GENERA AND SPECIES

Numerous spraying tests were made on a rather wide range of plants, including some that were only distantly related. Table I, which records the results of a test made on August 2, 1915, is fairly typical of the results obtained with these plants. It should be added, however, that in other tests sugar beet has not usually proven quite as susceptible as here indicated, being about the same as potato. Also, squash has usually proven a little more susceptible, being in about the same class as apple. Cabbage is distinctly the most resistant of the plants we have tested, though in the experiment recorded in this table cucumber was injured but little more.

TABLE I.—*Plants in field plots sprayed with calcium arsenite, 1.2 gm., and soap, 7.2 gm. per liter of water, to show the relative susceptibility of different species*

Name of plant	Injury.
Apple, Wealthy	Bad
Bean, White Navy	Very bad
Cabbage, Late Flat Dutch	Slight.
Cucumber, Improved Long Green	Do.
Potato, Early Ohio	Moderate.
Pea, Senator	Bad
Rutabaga, Monarch	Moderate.
Squash, Yellow Crookneck	Do.
Sugar beet, Klein Wenzlebener	Bad.
Tomato, Enormous	Moderate.
Turnip, White Globe	Do.

We suggest a table of susceptibility of plants we have sprayed, as shown in Table II. In using this table one must keep in mind that different varieties of the same crop vary somewhat among themselves in this respect. In this table an attempt is made to range the plants from the most resistant to the most sensitive.

TABLE II.—*Approximate order of foliage susceptibility to arsenical injury*

1. Cabbage.	6. Tomato.	11. Pea.
2. Sugar beet.	7. Rutabaga.	12. Squash.
3. Potato.	8. Turnip.	13. Cucumber.
4. Apple.	9. Cherry.	14. Peach.
5. Pear.	10. Plum.	15. Bean.

Something about the condition of plants of the same kind seems to vary at times to make them more or less susceptible. This accounts for some apparent discrepancies between Tables I and II. Repetition for confirmation is recognized as of fundamental importance in all scientific experiments, but is indispensable in studies of arsenical injury.

TABLE III.—*Fruit trees sprayed with copper aceto-arsenite and with calcium arsenite to show relative susceptibility of different species*

Name of tree.	Injury.	
	Copper aceto-arsenite 2.4 gm. to 1 liter of water.	Calcium arsenite 1.2 gm. to 1 liter of water.
Apple, McIntosh.....	Very slight.....	Moderate.
Crab apple, Transcendent.....	None.....	Do.
Cherry, Montmorency.....	Moderate.....	Very bad.
Cherry, Royal Ann.....	Slight.....	Nearly defoliated.
Pear, Flemish Beauty.....	Very slight.....	Moderate.
Plum, Bradshaw.....	Very bad.....	Defoliated.

For the variety used, the order of injury here indicated is plum, cherry, pear, apple, and crab, of which the plum is distinctly most susceptible. From the conflicting reports of other investigators it seems likely that this order will vary according to the variety used. We made no tests on the peach, as no trees were available, but it is quite generally conceded that it is more tender than any of the fruits named in Table III.

Roses were sprayed several times for a comparison with apple. Harrison's yellow rose seemed about like Okabena apple in susceptibility, and the Rugosa a little more tender.

#### DIFFERENCES IN VARIETIES OF THE SAME SPECIES

It is important to know if arsenical spray injury on any variety that happens to be chosen will be the same on other varieties under similar conditions. Among apples it is quite generally conceded that Ben Davis is especially susceptible, though strictly comparative data are not abundant in the literature.

In our work we have had many opportunities for comparing susceptibility of apples, and we have thoroughly tested Alexander, Ben Davis, Charlottenthaler, Gano, Hybernal, Lieby, McIntosh, McMahon, Okabena, Oldenburg, Rome, Shiawassee, Wagener, Tetofsky, Yellow Transparent, Greenwood, Crab, Hyslop crab, and Transcendent crab. Of these the crab apples have shown themselves distinctly more resistant than the standard apples, especially the Transcendent, which is almost in a class by itself. Among the standard varieties Ben

Davis, Gano, and Tetofsky have been conspicuously susceptible, while the others have shown no very consistent differences. They are so nearly alike that in one test certain ones would be injured a little more than the rest, while in other tests other varieties would be most injured. For practical purposes they may be considered about alike. It seems probable that this varietal susceptibility of apple trees is due to the thickness or character of the cuticle on the leaves rather than to a difference in the living cells within, for we have shown (30, p. 304) that when fresh wounds through the bark are treated with arsenical compounds, Ben Davis is not more injured or Transcendent less injured than other varieties.

Of the common garden beans, four varieties were compared. These were the White Navy, Red Kidney, Dwarf Horticultural, and Burpee Stringless. In their susceptibility to calcium arsenite, copper acetoarsenite, and London purple they were almost identical.

From our experiments and those of Woodworth (35) we may conclude that among plants of different species, and different varieties of the same species, few general rules may be laid down, and each species and variety must be actually tested in comparison with others to know its susceptibility. To be sure, some groups, as beans, are especially susceptible (probably all of them) and probably all cabbages are relatively resistant. But among plants of intermediate susceptibility such as apple, cherry, rose, potato, and sugar beet, varietal difference is sufficient to make it unsafe to draw comparisons between these species without stating the varieties.

#### INDIVIDUAL DIFFERENCES

Do individuals of the same species and variety show differences in susceptibility? If such be the case, general conclusions can not be drawn with safety from single tests, and some repetition is necessary to get the prevailing tendency, the amount of repetition depending upon the degree and frequency of individual variation and the exactness of the method used.

In carrying on this investigation, practically all the herbaceous plants used, whether in the greenhouse or in the field plots, were sprayed in triplicate and in some cases three branches of as many different orchard trees were sprayed in the same way. Opportunity was thereby offered for a very large number of comparisons. The results showed that the tendency to individual variation is quite marked. As this tendency is a matter of degree and we have no standards for expressing it, reference may be made to Table IV, which is quite representative of this condition. (See also Tables IX to XIV.) Doubtless this variation is partly apparent and partly real. With the greatest of care it is difficult to keep a large number of plants under identical conditions after spraying. One may be kept in a little more shaded place than another, or have better air circulation, or be brushed more in working among the plants. This difficulty is even greater in treating branches of orchard trees. Using every precaution to eliminate such factors, individual differences still appeared sufficient in degree to convince one that the triplicate plants at the time of spraying were not always alike in susceptibility. We believe that this is due in part to the environmental conditions under which the plants were grown, though all showing visible abnormalities were rejected before spraying, and there also may be a natural tendency to more or less resistant strains.



Nevertheless, the investigator in this field must give it due recognition and repeat all work until a predominant tendency is clearly established. We have made it a point, therefore, in all cases in which certain results shown in a published table differed from those most commonly observed under the same conditions, to call attention to this fact.

TABLE IV.—*Tomato plants in greenhouse, sprayed in triplicate, showing individual differences in susceptibility*

Chemical.	Amount of chemical in 2 liters of water.	Amount of soap in 2 liters of water.	Injury.		
			Plant 1.	Plant 2.	Plant 3.
Lead arsenate, di-plumbic, Baker	Gm.	Gm.			
	9.4	0	None	None.	None.
	9.4	7.2	do	do	Do.
	9.4	14.4	do	do	Do.
	9.4	28.8	Moderate	Moderate	Moderate.
Calcium arsenate, Merck.	2.8	0	Very slight	Very slight	Very slight.
	2.8	7.2	Moderate	Slight.	Slight.
	2.8	14.4	Slight	do	Do
	2.8	28.8	Moderate	Moderate.	Moderate.
Calcium arsenite, Adler	2.4	0	Moderate	Moderate	Moderate
	2.4	7.2	Bad.	Bad	Bad.
	2.4	14.4	Very bad	Very bad	Very bad.
	2.4	28.8	Partly defoliated.	Partly defoliated.	Partly defoliated.
Calcium arsenite, Baker	2.4	0	Moderate	Moderate	Moderate
	2.4	7.2	Very bad	Very bad.	Very bad
	2.4	14.4	do	do	Do.
	2.4	28.8	do	do	Do.
Calcium arsenite, Merck.	2.4	0	Slight	Slight	Slight.
	2.4	7.2	do	Moderate.	Do.
	2.4	14.4	Very slight	Very slight.	Very slight.
	2.4	28.8	do	Slight.	Moderate.
Arsenic trisulphide, Baker.	0.8	0	None	Slight	Very slight.
	.8	7.2	Slight	do	Moderate.
	.8	14.4	Moderate	Moderate	Slight.
	.8	28.8	Very bad	Very bad.	Very bad.

#### DIFFERENCES IN PARTS OF THE PLANT

In the spraying of trees and other plants all aerial portions are necessarily coated. This may or may not be necessary from the standpoint of controlling insects and diseases, but as it is practically unavoidable it makes the relative susceptibility of different parts of the plant to injury by the spray mixture of much importance in all spraying operations. This is especially true of fruit trees where, for example, the fruit may be russeted by Bordeaux mixture, greatly reducing its market value, while the same amount of burning on the leaves is unimportant.

## STEMS, FRUIT, AND FOLIAGE

On all kinds of plants sprayed in this investigation the leaves were the most susceptible portion. Most of the herbaceous plants bore no fruit prior to the time of spraying, but beans, cucumbers, and squashes were, in a few cases, exceptions. On apple trees the leaves proved much more tender than either stems or fruits. This is true to such a marked degree that any arsenical treatment that caused even the slightest direct damage to fruit or bark nearly defoliated the sprayed branch. So far as this fruit is concerned, therefore, and probably in general, arsenical spray injury is a problem of foliage injury almost exclusively. The apple fruit proved slightly more susceptible to very severe treatment than the most tender portion of the bark on the stem.

We have frequently noted that with plants of all kinds, whether herbaceous or woody, the youngest portion of the stem is the most easily injured. With leaves, however, this is not the case. Old leaves nearly ready to fall are damaged most, while young ones just expanding are most resistant.

On the apple the foliage injury takes place almost exclusively through the lower epidermis. Gillette (10) sprayed the upper surface and then both surfaces by way of comparison and found that—

wherever this was done, the damage sustained by the leaves that were wet on both sides was fully double that sustained by those wet only on the upper surface.

Woodworth (35) tried painting the leaves with white arsenic, Paris green, and London purple, some on one surface, some on the other, and some on both. The entire surface was coated. He concluded that the lower side is more susceptible than the upper, the difference in his experiments being in the proportion of 47 to 22.

In our own work the arsenical was applied individually to many leaves with a very soft brush, some to the upper side and others to the lower side. It was found that if the painted area extended to the margin it was practically impossible to keep a little of the liquid from extending over onto the opposite surface from that intended. Therefore, after a few preliminary experiments the practice was followed by painting a strip about  $\frac{3}{4}$  by  $1\frac{1}{4}$  inches down one side of the midrib. This strip never approached the leaf margin. In Table V each report represents the injury of 10 painted leaves on one shoot. Both sodium arsenite and calcium arsenite were used in this experiment, one being a soluble and the other a nearly insoluble compound. Soap was added in some cases to cause the liquid to spread better and to penetrate the pubescence on the leaf more readily.

The results shown in Table V are quite typical and indicate that the arsenical injury to apple foliage from spraying is brought about largely by absorption from the lower surface. Indeed, in some of our experiments when very toxic solutions were used no trace of injury occurred on leaves treated on the upper surface. We are led to believe that in those cases where injury did occur after treatment of the upper surface, there was some tiny abrasion, not easily visible to the naked eye. In view of our results it is difficult to account for so much injury from treating the upper surface as reported by Woodworth (35). The only essential difference in method was that he painted the whole surface instead of the central portion only. When we did the same we could not readily avoid getting some of the liquid over the margin onto the

other side with consequent injury and this may explain the discrepancy. Also, in his experiments the epidermis treated may not have been so free from tiny abrasions.

TABLE V —Leaves of apple treated on the upper or the lower surface to determine the relative injury

Variety <sup>1</sup>	Chemical.	Strength.	Injury.	
			Upper.	Lower.
Transcendent Wealthy McIntosh Yellow Transparent	Calcium arsenite.	1-1,000	{Very slight . . . None . . . . . Very slight None . . . . .	Very slight. Very bad. Moderate. Very slight
Transcendent Wealthy McIntosh Yellow Transparent	Calcium arsenite plus soap.	1-1,000 (14.4 gm. in 2 li- ters).	{None . . . . . do . . . . . do . . . . . do . . . . .	Very slight. Do Do. None.
Transcendent Wealthy McIntosh Yellow Transparent	Calcium arsenite.	1-500	{Very slight . . . None . . . . . Very slight . . . None . . . . .	Slight. Do. Very bad. Slight.
Transcendent Wealthy McIntosh Yellow Transparent	Calcium arsenite plus soap.	1-500 (14.4 gm. in 2 liters).	{None . . . . . do . . . . . do . . . . . do . . . . .	Slight. Moderate. Very slight. Partly defoliated.
Transcendent Wealthy McIntosh Yellow Transparent	Sodium arsenite.	1-1,000	{None . . . . . Very slight . . . None . . . . . Very slight . . .	Moderate. Very bad Bad. Do.
Transcendent Wealthy McIntosh Yellow Transparent	Sodium arsenite plus soap.	1-1,000 (14.4 gm. in 2 li- ters).	{None . . . . . do . . . . . Very slight None . . . . .	Slight. Moderate. Partly defoliated. Defoliated.
Transcendent Wealthy McIntosh Yellow Transparent	Sodium arsenite.	1-500	{Slight . . . . . None . . . . . Moderate . . . . Slight . . . . .	Bad. Very bad. Partly defoliated. Very bad.
Transcendent Wealthy McIntosh Yellow Transparent	Sodium arsenite plus soap.	1-500 (14.4 gm. in 2 liters).	{Very slight . . . None . . . . . Slight . . . . . None . . . . .	Very bad. Partly defoliated. Do. Defoliated.

Whether the under surface of apple leaves is more susceptible to injury because of the greater prevalence of stomata or because of a thinner or more permeable cuticle is yet to be determined. We are inclined to the latter view.

To determine if this greater protection of the upper epidermis is found also in other plants the leaves of a dozen different shrubs and trees on the college campus were painted with sodium arsenite and soap in different strengths. The procedure was the same as described for apple on page 515. These plants are as follows: Alder, barberry (common, green), birch (white), box elder, buckthorn (English), chokecherry, dog-

wood, lilac (purple), lilac (white), maple (Norway), Siberian pea tree, willow (golden). Repeated tests showed that in all species tested the upper epidermis is decidedly more protective against arsenical injury than the lower.

#### RELATIVE INJURIOUSNESS OF ARSENICAL CHEMICALS

When a number of arsenical compounds are sprayed upon plants the relative injury done by them may be influenced by two factors, (1) their relative solubility and (2) their relative toxicity to the plants treated. It so happens that with reference to solubility, arsenical compounds fall into two fairly well defined groups, those that dissolve readily in a relatively small amount of cold water and those that are only slightly soluble in cold water. The former we will for convenience designate as the soluble arsenical compounds and the latter as the insoluble arsenical compounds, it being fully recognized that a small amount goes into solution while the greater part remains undissolved when used in spraying work.

#### READILY SOLUBLE ARSENICAL COMPOUNDS

Compounds of this class can not be used for spraying plants to control insect pests owing to the injury done when enough is applied to kill the insects. Their practical significance in this connection lies in their utility as weed killers and in the possibility of their presence as impurities in the so-called insoluble arsenical insecticides. Their toxicity may be compared on either of two bases, (1) using equal parts by weight of the different chemicals or (2) such amounts of them as will contain equal amounts of arsenic. Our experiments included tests on both these bases.

The results shown in Tables VI and VII correspond very well with those that were usually found in the many tests of these compounds. It will be noted that on the basis of toxicity whether equal weights of the chemicals be used per liter or amounts to contain equal weights of arsenic, these compounds may be divided into three groups; cacodylic acid and the cacodylates are most toxic, the arsenites and arsenic acid next, and the arsenates least. Ammonium arsenate constitutes one exception, as it causes injury equal to the arsenites. Ammonium arsenite was used a little, but is unsatisfactory owing to its instability.

TABLE VI.—*Apple sprayed with soluble arsenical compounds using the same number of grams for 2 liters*

Chemical.	Injury.		
	Concentration 1 to 10,000	Concentration 1 to 5,000	Concentration 1 to 1,000
Acid, arsenic.....	None . .	Slight . .	Bad.
Acid, cacodylic.....	Moderate.	Moderate . . .	Very bad
Ammonium arsenate.....	None . .	do . .	Bad.
Potassium arsenate.....	.do . .	None	Moderate.
Potassium arsenite.....	.do . .	Very slight	Bad.
Potassium cacodylate.....	.do . .	Moderate	Very bad.
Sodium arsenate.....	.do . .	None . . .	Slight.
Sodium arsenite.....	.do . .	Very slight	Bad.
Sodium cacodylate ..	.do . .	Moderate . .	Very bad

TABLE VII.—*Apple and tomato sprayed with soluble arsenical compounds in amounts to contain 7.5 gm. of arsenic in 2 liters of solution*

Chemical.	Injury.	
	Apple.	Tomato.
Acid, arsenic	Very bad	Very bad
Acid, cacodylic	Defoliated.	Partly defoliated.
Ammonium arsenate	Partly defoliated	Very bad.
Potassium arsenate	Bad	Bad
Potassium arsenite	Partly defoliated	Very bad.
Potassium cacodylate	Defoliated	Partly defoliated.
Sodium arsenate	Moderate	Bad
Sodium arsenite	Partly defoliated	Very bad.
Sodium cacodylate	Defoliated	Nearly defoliated.

Cacodylic acid and the cacodylates probably never occur in insecticides and are too expensive to be used as weed killers, and are therefore of more theoretical than practical interest. The scientific interest which they have in this connection lies chiefly in their very high toxicity to higher plant life and relative low toxicity to higher animal life. Cushny (6) states:

The action being due to the ion and not to the element . . . organic arsenic combinations in which the metallic atom is directly attached to the carbon atom are only feebly poisonous. The earliest of these is sodium cacodylate,  $(\text{CH}_3)_2\text{AsOONa}$ .

Merck's Index (20) states also:

The cacodylates (which see) are now largely used instead of the alkali arsenites, as the former are far less toxic.

It has been stated on high authority (23) that—

Arsenious acid is extremely poisonous, whereas many, both of higher and of the lower plants, can withstand large doses of arsenic acid and can accumulate large quantities of arsenic when supplied to them in this form

This statement, while perhaps true under some conditions, is wholly misleading if applied to spray mixtures.

#### NEARLY INSOLUBLE ARSENICAL COMPOUNDS

Turning to the arsenical compounds that are but slightly soluble, we find the comparison more difficult. In the first place it is hard to get these chemicals that are pure and of definite composition, and in some instances they are not fully named. For example, a "lead arsenate" may be diplumbic ortho-lead arsenate or triplumbic ortho-lead arsenate or a mixture of the two, and neither the label nor the statement of the manufacturers reveals which of the three it is. Indeed, it is doubtful if an absolutely pure diplumbic or triplumbic lead arsenate is now on the market. Then, too, some of these chemicals are so slightly injurious that they cause no injury except under such conditions as will permit the more injurious ones to nearly or quite defoliate the plants or branches, making fine comparisons difficult. Furthermore, the number of chemicals in this group is so large, especially if we use several brands of the more important ones, that no single series of comparative tests is likely to be reliable throughout, owing to individual differences in the plants used, unless several are sprayed at the same time with each mixture,

making a series so large that the last must be applied much later than the first. Table VIII will, however, give a nearly correct idea of their relative injuriousness. Under the conditions governing these experiments it should be constantly kept in mind that these results might be different if other brands of the chemicals were used or if climatic conditions were different.

If we consider the results shown in this table and also those from many other spraying tests, we come to the conclusion that the order of injuriousness of the chemicals listed in Table VIII when sprayed on to foliage under our conditions is as follows:

1. Calcium arsenite.
2. Arsenic trisulphid.
3. Barium arsenate.
4. Calcium arsenate.
5. Lead arsenite.
6. Copper aceto-arsenite.
7. Arsenic disulphid.
8. Arsenic trioxid.
9. Zinc arsenite.
10. Lead arsenate diplumbic.
11. Lead arsenate triplumbic.
12. Ferrous arsenate.

TABLE VIII — *Results of spraying different plants with "insoluble" arsenical compounds in such amounts as to give equal weight of arsenic to 2,000 cc. water to show relative toxicity*

Chemical	Injury to bean, May 3, 1915 (1 gram arsenic in 2 liters of water)	Injury to apple, June 18, 1915 (2 grams arsenic in 2 liters of water)	Injury to tomato, Aug. 14, 1916 (2 grams arsenic in 2 liters of water)
Arsenic trioxid, Baker	Very slight.	None	None.
Arsenic disulphid, Merck	do	do	Do
Arsenic trisulphid, Baker	Bad	Slight	Very bad
Barium arsenate, Adler	Moderate	Moderate	Bad
Calcium arsenate, Merck	do	do	Moderate
Calcium arsenite, Baker	Defoliated	Partly defo- liated.	Partly defo- liated.
Ferrous arsenate, Merck	Very slight.	None	None.
Lead arsenate, diplumbic, Baker	do	do	Do
Lead arsenite, Merck	do	do	Very slight
Lead arsenate, triplumbic, Baker	None	do	None
Paris green, Baker	Moderate	Moderate	Do.
Zinc arsenite, "Ortho 40"	Very slight.	Slight	None

Probably the most striking fact brought out by this phase of the work is that arsenic trioxid may be applied with so little injury. It is well known that this compound has been kept out of general use by the injury it has caused under field and orchard conditions in other localities. We have sprayed both apple and several herbaceous plants many times and on many dates during a period of several years and have never found it especially injurious if the mixture was applied as soon as prepared. This might not be true in localities with a high humidity. Even the freshly prepared amorphous form which is more soluble than the crystalline form has given less injury than the majority of the slightly soluble arsenical

compounds, and indeed it was scarcely more injurious than the crystalline form.

On the other hand, it seems surprising that calcium arsenite could have remained in use as an insecticide for so long as it did. It has gradually been abandoned, partly because of its burning of the foliage and partly because some time is consumed in its preparation according to the Kedzie (Woodworth and Colby (37)) or the Kilgore (14) formulas, which are the ones most generally followed. We have tried three different brands, one of them especially prepared for our use, besides the home-made articles prepared after the Kedzie and the Kilgore formulas, and are left no other alternative than to place it at the head of the list of slightly soluble arsenical compounds, considered from the standpoint of injurious properties. Indeed, used in strengths to contain equal amounts of arsenic, the home-made calcium arsenites were even more harmful than the Baker brand on which we based most of our work.

To determine the variability of compounds supposed to be identical, tests were made with different brands of calcium arsenite, lead arsenate, zinc arsenite, and arsenic trioxid, using four brands of calcium arsenite, 4.9 gm. to 2 liters of water, and applying to apple foliage. Of the four, one<sup>4</sup> caused in a typical case moderate injury; the second,<sup>5</sup> very slight; the third,<sup>6</sup> bad; and the fourth,<sup>7</sup> very slight. In this case there was no consistent relation between the arsenic content of the different brands and the amount of injury produced.

More than 30 brands of lead arsenate were tried. Some were supposed to be diplumbic, others triplumbic, and others mixtures of the two, while still others gave no clue without chemical analysis as to their kind. Some were paste and others were dry. Some were prepared for use as insecticides and others as pure chemicals for technical use. It is doubtful if the results of these tests merit publication in tabular form. Marked differences were found, to be sure, but as some factories do not put out a uniform product, and as others have changed their processes of manufacture since the tests were made, and still others will doubtless do so in the near future, such a list could not safely be used as a basis for selecting lead arsenates for orchard spraying. The same could, perhaps, be said also of the calcium arsenites and zinc arsenites discussed above. The important point is that lead arsenates do vary in composition, as indicated by their analyses and by their injurious properties, even though the labels would not indicate the fact.

It is generally believed that diplumbic lead arsenate has a greater tendency to injure foliage than the triplumbic form. This is doubtless true in a general way, but we have had some brands of the diplumbic that were just as safe to use as most of the brands of triplumbic arsenate.

Four lots of arsenic trioxid were compared, a crystalline form and an amorphous form from one manufacturer,<sup>8</sup> a crystalline form from another,<sup>9</sup> and a crystalline form taken direct from the arsenic retainer of a smelter<sup>10</sup> situated at Anaconda, Mont. In addition, an arsenic trioxid paste was made from each of these four by grinding the dry powder in a mortar with a little water. This was allowed to stand a week or more before using. The injury caused by these various lots of arsenic trioxid was practically equal.

<sup>4</sup> Adler's.

<sup>5</sup> California Spray Co.'s "Ortho 40."

<sup>6</sup> Sherwin-William's.

<sup>7</sup> Thomson's.

<sup>8</sup> J. T. Baker.

<sup>9</sup> Merck.

<sup>10</sup> The Washoe.

## EFFECTS OF ADDING OTHER INSECTICIDES, LIME, ETC.

In orchard, field, and garden practice it is often desired to add various substances to arsenical spraying mixtures. These substances are of various kinds: Other insecticides, or fungicides for combination sprays, soap, or other colloidal substances for an adhesive or "spreader" on such plants as cabbage and sugar beet to retard the settling of the arsenical suspension, as recommended by Parker (22), or various materials to lessen the tendency to burn the foliage.

## LIME SULPHUR

The addition of lime sulphur to kill scale insects or to prevent apple scab is often desirable. This substance brings about a chemical reaction with most arsenical insecticides, and it is of interest to know whether the insecticidal and fungicidal properties of either or both are destroyed and whether the tendency to burn the foliage is increased or decreased. The first of these questions has been quite thoroughly discussed by Cordley (4) and Melander (18) and is hardly within the province of this paper. The effect of lime sulphur on the injury caused by the different arsenicals is shown in Table IX. The tests from which this table was prepared ran through three seasons and were carried on in four different parts of the State. Several brands of the chemicals were used. The arsenicals were used in such strengths as to contain either 1 or 2 grams of arsenic for each 2 liters of water. The lime sulphur (factory boiled) was added at the rate of 77 cc. of 28° Baumé in every 2 liters of mixture. If in any case the lime sulphur (control) alone caused injury, the series was rejected, but this was extremely rare.

TABLE IX.—*Effects of lime sulphur on the burning properties of various arsenical compounds*

Arsenical compounds to which lime sulphur was added	Number of brands.	Number of applications.	Number of times injury increased.	Number of times injury decreased.	Number of times the same	Number of times no injury with or without.
Arsenic trioxides . . . . .	2	3	2	0	1	0
Arsenic disulphid . . . . .	1	2	2	0	0	0
Arsenic trisulphids . . . . .	2	4	3	0	1	0
Barium arsenate . . . . .	1	1	0	0	1	0
Calcium arsenites . . . . .	3	16	2	9	3	2
Copper aceto-arsenite . . . . .	2	20	12	2	2	4
Ferrous arsenates . . . . .	3	8	0	1	0	7
Lead arsenates, triplumbic . . . . .	8	52	25	0	1	26
Lead arsenates, diplumbic . . . . .	13	85	36	0	0	49
Lead arsenates, mixtures . . . . .	10	27	15	0	1	11
Zinc arsenites . . . . .	6	42	10	3	2	27

From Table IX it may be seen that lime sulphur causes an increase in the injury produced by most arsenical compounds. This is especially true of copper aceto-arsenite, the lead arsenates and zinc arsenite. With calcium arsenite, however, it usually causes a reduction in the amount of injury, though not to such an extent as to make this compound



safe to use. Even with lime sulphur added calcium arsenite does more damage to the foliage than any other arsenical in the list given in Table IX.

We have given some attention to finding an explanation for the reduced injury by calcium arsenite when lime sulphur is added. As a principle of physical chemistry, the lime sulphur containing calcium sulphids, which are more readily soluble than calcium arsenite, would be expected to prevent to some extent the ionization of the less soluble salt. If this were the only consideration involved, other soluble calcium salts would be expected to have the same effect. To test this experimentally we added calcium nitrate to certain lots of calcium arsenite suspension, and calcium chlorid to other lots. These were sprayed upon tomato, bean, and cucumber plants in comparison with calcium arsenite alone and calcium nitrate and calcium chlorid alone. In almost every instance the injury was increased slightly by the addition of the nitrate and chlorid, though these compounds in themselves caused no injury. After repeated tests we decided that other factors than the restraint of ionization must enter in. This question is now receiving further study.

#### TOBACCO EXTRACT

This insecticide has come into quite general use for the control of plant lice. It will often save the labor of one spraying application to combine it with an arsenical spray mixture. The results of such combination in injury to the foliage are shown in Table X. The conditions under which the tests there recorded were carried on as to dates, localities, arsenical chemicals, etc., are essentially as described on page 521. The tobacco extract is the "Black Leaf 40." It was used at the rate of 2.5 cc. to each 2 liters of water.

It is evident from Table X that the effect of tobacco extract upon the injurious properties of the arsenical compounds is not marked. It is probable that we may safely state that it does not increase the injury with any of the arsenicals tested, to such an extent as to make its use undesirable and on the other hand it does not decrease the injury in any case enough to make this an important consideration.

TABLE X.—*Effects of tobacco extract on the burning properties of various arsenical compounds*

Arsenical compounds to which tobacco extract was added.	Number of brands	Number of applications.	Number of times injury increased	Number of times injury decreased.	Number of times injury was the same.	Number of times there was no injury with or without the extract.
Arsenic trioxids . . . .	2	2	0	0	1	1
Arsenic disulphid. . . .	1	1	1	0	0	0
Arsenic trisulphids. . . .	2	4	0	1	1	2
Barium arsenate. . . . .	1	2	2	0	0	0
Calcium arsenites. . . . .	3	22	3	7	9	3
Copper aceto arsenites . . .	2	20	2	6	4	8
Ferrous arsenates . . . . .	3	15	1	0	1	13
Lead arsenates, triplumbic. . .	7	62	3	2	0	57
Lead arsenates, diplumbic . .	13	97	5	3	2	87
Lead arsenates, mixtures. . .	10	28	2	0	0	26
Zinc arsenites. . . . .	6	52	8	3	10	31

## SOAP

In spraying some plants, for example, cabbage, and to a less extent sugar beet, the spray mixture does not spread on the surface of the leaf and adhere, but rolls off in droplets, leaving most of the surface unprotected. It has been found that the addition of soap will nearly or quite obviate this. Furthermore, Parker (22) has shown that soap will very materially retard the settling of lead arsenate from its suspension in water. The natural tendency of soap to increase the solubility of the slightly soluble arsenical compounds is well recognized and the question arises, will this result in an increased tendency to injure the foliage on the part of some or all of the arsenical insecticides?

During this investigation several different kinds of soap were tried and several strengths were used, but in the tests recorded in Tables XI and XII one brand<sup>11</sup> was used exclusively, 14.4 gm. being added to each 2 liters of spray mixture. The arsenical chemicals were the same in kind and strength as recorded on page 521.

TABLE XI.—*Effects of soap on the burning properties of various "insoluble" arsenical compounds*

Arsenical compounds to which soap was added.	Number of brands	Number of applications	Number of times injury increased	Number of times injury decreased	Number of times injury was the same	Number of times there was no injury with or without the soap
Arsenic trioxides	4	105	16	7	11	71
Arsenic disulphids ..	1	14	2	0	0	12
Arsenic trisulphids	2	17	9	1	3	4
Barium arsenates	1	9	4	1	4	0
Calcium arsenates	1	77	48	6	17	6
Calcium arsenites ..	3	123	27	28	63	5
Copper aceto arsenites	2	123	8	64	28	23
Ferrous arsenates	3	29	1	0	0	28
Lead arsenates, triplumbic.	8	95	13	1	0	81
Lead arsenates, diplumbic ..	13	140	56	0	1	83
Lead arsenates, mixtures...	10	57	19	0	0	38
Lead arsenite ..	1	5	4	0	0	1
Zinc arsenites	6	90	11	10	17	52

Table XI shows that of the "insoluble" arsenicals tested all but three cause greater injury if soap is added. This increased injury is most conspicuous in the lead arsenates. Of these, 8 brands were triplumbic, 13 diplumbic, and 10 were probably mixtures of both. The increase of injury through the addition of soap is a little more pronounced with the diplumbic than with the triplumbic arsenates, but the difference is not so great as some have supposed.

One of the most surprising observations made in this phase of the investigation is the reduction in injury caused by copper aceto-arsenite when soap is added.

Not all brands of soap give the same results when combined with arsenical spray mixtures. Some appear to retard the injury by copper aceto-arsenite more than others, and they vary in the degree to which they increase the injury by lead arsenates and other compounds. Neither do they show equal power to keep lead arsenate in suspension. While

<sup>11</sup> "Diamond C"

considerable work was done with different soaps, 18 in number, the results were not sufficiently consistent and conclusive to warrant a detailed statement concerning them. It seems probable that the product of some of the soap factories is not uniform, and the number of brands on the market is great and the assortment found in different parts of the country varies so much that it seemed inadvisable to attempt to study them fully. However, it may at least be said that some quite consistently give bad results with the arsenicals and others are relatively harmless, while still others are variable. The brand most used in this investigation<sup>12</sup> was rather consistent and intermediate in its effect upon the arsenicals.

Soap appears to have some effect also upon the injurious properties of the soluble arsenical compounds, as shown in Table XII. This tendency is in general toward reduction when soap is added, but it is not so marked as to have great significance. Perhaps the more even spread on the leaf surface would account for it.

TABLE XII.—*Effects of soap on the burning properties of soluble arsenical compounds*

Soluble arsenical compounds to which soap was added.	Number of applications.	Number of times injury increased.	Number of times injury decreased.	Number of times injury was the same.	Number of times there was no injury with or without soap.
Arsenic acid.....	9	0	4	3	1
Ammonium arsenate.....	7	0	2	3	2
Cacodylic acid.....	3	0	0	3	0
Potassium arsenate.....	7	0	4	0	3
Potassium arsenite.....	8	1	3	4	0
Potassium cacodylate.....	5	2	1	1	1
Sodium arsenate.....	10	0	3	3	4
Sodium arsenite.....	9	1	2	4	2
Sodium cacodylate.....	5	0	1	2	2

#### LIME-SULPHUR AND TOBACCO EXTRACT

It may be at times desirable to add both lime sulphur and tobacco extract to lead arsenate or other arsenical spray mixtures, provided the efficiency of neither is destroyed and the resulting mixture is not dangerous to foliage.

TABLE XIII.—*Effects of lime sulphur and tobacco extract on the burning properties of various arsenical compounds*

Arsenical compounds to which lime sulphur and tobacco extract were added.	Number of brands.	Number of applications.	Number of times injury increased.	Number of times injury decreased.	Number of times injury was the same.	Number of times there was no injury with or without soap.
Arsenic trioxides.....	1	2	0	0	0	2
Calcium arsenites.....	1	10	0	7	2	1
Copper aceto-arsenite.....	1	16	7	1	4	4
Ferrous arsenites.....	1	2	1	0	0	1
Lead arsenates, triplumbic.....	4	38	19	0	0	19
Lead arsenates, diplumbic.....	9	58	25	1	0	32
Lead arsenates, mixture.....	6	12	12	0	0	0
Zinc arsenite.....	2	30	10	4	0	16

<sup>12</sup> "Diamond C."

A comparison of Tables IX and XIII leads to the conclusion that the addition of lime-sulphur and tobacco extract to arsenical spray mixtures has practically the same effect upon the foliage as lime sulphur alone.

Arsenic trisulphid may constitute an exception, but our tests with this compound combined with both tobacco extract and soap are too few to be conclusive. It is doubtful, however, if the trisulphid will ever come into general use as an insecticide.

#### LIME SULPHUR, TOBACCO EXTRACT, AND SOAP

This combination with arsenical compounds is not a desirable one as it often forms a curdled mass that is difficult to apply to the foliage. It also has a greater tendency to injure the foliage than any other combination we have tried. Calcium arsenite in combination with these three substances is the only exception, as may be seen by Table XIV.

TABLE XIV.—*Effects of lime-sulphur, tobacco extract, and soap upon the burning properties of various arsenical compounds*

Arsenical compounds to which lime-sulphur, tobacco extract, and soap were added	Number of brands	Number of applications.	Number of times injury increased	Number of times injury decreased.	Number of times injury was the same.	Number of times there was no injury with or without soap.
Arsenic trioxides.	2	5	3	1	0	1
Arsenic disulphid.	1	2	2	0	0	0
Arsenic trisulphids.	2	5	5	0	0	0
Barium arsenate.	1	1	0	0	1	0
Calcium arsenites.	3	17	2	8	4	3
Copper aceto-arsenites	2	20	12	2	3	3
Ferrous arsenates	3	8	2	1	0	5
Lead arsenates, triplumbic	8	51	29	0	0	22
Lead arsenates, diplumbic	13	84	57	0	0	27
Lead arsenates, mixtures	11	30	26	0	0	4
Zinc arsenites	6	40	17	1	1	21

#### GELATIN, AGAR, AND MILK

While soap is probably the best material known to make spray mixtures spread on leaves of cabbage, sugar beets, etc., where there is a tendency to roll off the smooth or waxy surface, its tendency to increase injury by most arsenical compounds gives an incentive to search for some other spreader. Moore (21) has given a very thorough discussion of the principles and practices of spreaders in spraying work, and a full bibliography of the subject. Gelatin, agar, and separated milk, having possibilities along this line, were tested in our work with various arsenical compounds to determine if they would influence the burning.

The gelatin was used in strengths of 0.1 per cent and 0.4 per cent (i. e., 1 and 4 grams to the liter). The agar was used in strengths of 0.01 and 0.04 per cent. The separated milk was used in strengths of 0.1 per cent and 0.4 per cent. Various arsenical spray mixtures, including lead arsenate, Paris green, and calcium arsenite, were tried singly and in combination with these three "spreader" both on greenhouse plants and in the field, and also in the orchard. Repeated experiments showed no tendency on the part of these materials to increase the arsenical injury, and while they are not so efficient as soap in pro-

moting the adhesion of the mixture, yet they may be regarded as very serviceable. The agar in strengths used increased the spreading power least and would have to be used much stronger, perhaps 0.1 per cent, but gelatin 0.4 per cent and milk 0.4 per cent were quite satisfactory for sugar beets, though only moderately so for cabbage.

#### LIME

It has been recommended by Kilgore (14) that lime be added to Paris green and other arsenicals to restrain the injurious action on the foliage.

On tomatoes and beans in the greenhouse we have tested quite thoroughly unslaked lime with calcium arsenite and, with fewer repetitions, with lead arsenate (Corona) and with Paris green. Quite consistently the use of lime has very materially reduced the burning action of these three arsenical compounds as shown by Table XV.

TABLE XV.—Effect of lime in combination with arsenical compounds

Date.	Plant.	Chemical.	Amount in 2 liters	Lime	Injury.
			Gms.	Gms.	
July 25, 1917	Tomato.	Calcium arsenite.	1.9	None.	Bad.
Do	do	do	1.9	4.6	Do
Do	do	do	3.9	None	Defoliated
Do	do	do	3.9	4.6	Partly defoliated
Sept 3, 1917	do	do	2.0	None.	Moderate.
Do	do	do	2.0	9.0	Slight.
Do	do	do	3.9	None	Partly defoliated.
Do	do	do	3.9	9.0	Moderate
Mar. 3, 1918	Bean	Lead arsenate (Corona).	9.7	None.	Very slight
Do	do	do	9.7	9.7	None
Do	do	do	19.5	None.	Moderate.
Do	do	do	19.5	19.5	None.
Do	Tomato	do	9.7	None.	Slight.
Do	do	do	9.7	9.7	None.
Do	do	do	19.5	None.	Moderate
Do	do	do	19.5	19.5	None.
Sept 3, 1917	do	Paris green	6.9	None.	Bad.
Do	do	do	6.9	6.9	Slight.
Do	do	do	9.2	None.	Very bad.
Do	do	do	9.2	9.0	Slight.

#### FERROUS SULPHID

Volck (34) especially has advocated the use of ferrous sulphid to decrease the burning effect of zinc arsenite. We have used this compound (7.2 gm. to 2 liters) many times in combination with different brands of calcium arsenite and of zinc arsenite and have found no general benefit in restraining the injury to the foliage. Usually the injury with and without the ferrous sulphid was the same, but occasionally it was a little more or a little less if the iron salt was added.

#### EFFECTS OF LETTING MIXTURES STAND BEFORE APPLYING

In the case of that group of arsenicals that are but slightly soluble in water and are often called "insoluble," one would naturally expect that if the suspension were applied as a spray as soon as mixed the amount

in solution would be less than if allowed to stand for some time and the injury correspondingly less. To what extent this principle will operate can be determined by experiment only.

To this end we have made repeated trials with arsenic trioxide and a few with calcium arsenite, calcium arsenate, lead arsenate, and Paris green.

Quite without exception the burning by arsenic trioxide steadily increased as the mixture was allowed to stand. Table XVI illustrates a typical demonstration of this fact. In this case suspensions of arsenic trioxide<sup>18</sup> (2.6 gm. in 2 liters) were prepared at different intervals so spaced that all would be ready for spraying at the same time, having stood the time periods indicated in the left hand column of the table. These suspensions after mixing were kept in an incubator at blood temperature and were stirred at intervals of about six hours, day and night. At the time of spraying the suspensions were thoroughly shaken and a small sample removed and filtered through Swedish filter paper, and the filtrate analyzed. The remainder was sprayed upon tomato and bean plants in triplicate.

TABLE XVI.—*Effects of letting arsenic trioxid stand after mixing*

Time after mixing.	Arsenic trioxid in solution.	Injury.	
		Bean.	Tomato.
	<i>Per cent.</i>		
5 minutes . . . . .	0.001392	Slight . . . . .	None.
1 hour . . . . .	.002784	Moderate . . . . .	Do.
2 hours . . . . .	.006960	Very bad . . . . .	Slight.
4 hours . . . . .	.012520	Defoliated . . . . .	Moderate.
8 hours . . . . .	.016720	.. do . . . . .	Do.
12 hours . . . . .	.024128	... do . . . . .	Bad.
24 hours . . . . .	.032480	Dead . . . . .	Very bad.

Table XVI shows strikingly the progressive increase in burning by allowing arsenic trioxid to stand in suspension and the corresponding increase in the amount of the chemical that went into solution. Other experiments showed that this increase in injury as a result of standing continued for at least 48 hours and was strongly in evidence whether or not soap was added to the mixture. Kilgore (14) has shown that arsenic trioxid suspended in water continues to dissolve for 10 days.

The tendency of calcium arsenite, calcium arsenate, lead arsenate, and Paris green to increase in burning power as a result of standing in suspension is relatively slight.

#### EFFECTS OF REPEATED SPRAYING

It is often necessary for the control of insect pests to spray plants or trees two or more times at intervals of a few days or weeks, and in such cases it is important to know if the second or later applications are especially dangerous from the standpoint of injury to the foliage. Clinton and Britton (2) showed that the second application of zinc arsenite may do very serious damage, even though the first did little or no harm. Our work with two brands of zinc arsenite, Thomson's and "Ortho 40,"

<sup>18</sup> Baker's.

strongly confirms this observation. Quite uniformly the second application did much more damage than the first, and indeed a strength of 3.2 grams per liter, applied twice at intervals of about three weeks, did more harm than 6.4 grams applied once on either date. With calcium arsenite this tendency was noticeable but not nearly so pronounced as with zinc arsenite, while with lead arsenate and with copper acetoarsenite there was but little more injury after the second spray than after the first.

#### EFFECTS OF HUMIDITY

That humidity is one of the great contributing factors in arsenical spray injury is one of the most striking facts constantly in evidence throughout this investigation. This observation is by no means new and had already been noted by Gillette (11) and others; and certain manufacturers of lead arsenates have come to recognize the necessity of using safer mixtures on the Pacific coast and other humid regions than are required elsewhere.

Even with these facts in mind, however, we were almost astonished at the difference in results on leaves kept in moist and dry air. To demonstrate if possible the effects of humidity, an extensive series of spray mixtures was applied about simultaneously in different parts of the State, including an apple orchard near Flathead Lake (Mont.), where the humidity is generally relatively high. As it happened, however, this section was unusually dry during the course of the experiment and much more so than in the other localities where tests were made. Not a drop of rain fell for weeks and the dew point was not reached on a single night during the experiment. The days were rather hot, and wet and dry bulb thermometer readings made three times daily indicated a very low humidity every day and nearly every night. As a result almost none of the spray mixtures used caused any injury whatsoever. Even calcium arsenite (2 gm. in 2 liters of water) caused only slight injury. In the majority of cases, including all localities and dates, this compound in half this strength caused very serious injury to apple foliage, and sometimes defoliation.

A still more striking demonstration of the effects of humidity in extreme cases was made repeatedly by spraying tomato and other plants in pots and covering certain ones with bell jars and keeping duplicates outside. Under these conditions the moisture transpired by the leaves kept the air under the bell jars saturated.

The plants were all kept in diffused light in a basement laboratory to prevent rise of temperature under the bell jars as would happen if kept in direct sunshine. The plants were kept in this condition for usually one or two days and aerated twice daily. The bell jars were then removed and all plants placed under like conditions in the greenhouse until the notes were taken. Table XVII is representative of the results under these conditions.

Probably nothing in the whole series of experiments on arsenical spraying is more striking than the contrast of injury in dry air and in the saturated atmosphere under the bell jars, especially when soap is added. It might seem at first that the bell jar makes an extreme condition of humidity, but a saturated atmosphere out of doors is by no means unusual, especially near large bodies of water, as on the Pacific coast and in the Great Lakes region.

TABLE XVII.—*Showing the increased injury caused by a high humidity, procured by keeping sprayed tomato plants under bell jars*

Chemical.	Chemical in 2,000 cc.	Soap in 2,000 cc.	Injury.	
			Under bell jars.	Not under bell jars
	Gm.	Gm.		
Arsenic trioxid, Baker. . . . .	1.3	None.	Slight . . .	None.
Do. . . . .	2.6	None.	. . . do . . .	Do.
Calcium arsenite, Baker. . . . .	1.9	None.	Dead . . .	Moderate.
Calcium arsenite, Adler. . . . .	.6	None.	Very bad . .	None.
Do. . . . .	.6	14.4	. . do . . .	Do.
Lead arsenate, Baker, acid. . . . .	9.3	None.	None . . . . .	Do.
Do. . . . .	9.3	28.8	Moderate . . .	Do.
Lead arsenate, Baker, ortho . . . . .	12.0	None.	None . . . . .	Do.
Do. . . . .	12.0	28.8	Moderate. . . .	Do.
Lead arsenate (Corona). . . . .	9.3	None.	None . . . . .	Do.
Do. . . . .	9.3	14.4	Very bad. . . .	Do.
Copper aceto-arsenite, Baker. . . . .	2.4	None.	Nearly dead. . .	Do.
Do. . . . .	2.4	14.4	Very bad . . .	Very slight.

To determine if it makes a difference whether the plants were placed in the saturated atmosphere immediately after spraying or after drying a short time, some were put under bell jars for the first three days and then removed while others were not covered at once but inclosed the fourth, fifth, and sixth days; and likewise some were kept under bell jars the first day only, others the second day only, and still others the third day only. Reducing the time, in another experiment some were covered the first hour after spraying, others the third hour, others the first hour of the second day and still others the first hour of the fifth day. The results showed that a saturated atmosphere for even so short a period as one hour considerably increased the injury, though a longer time caused still more damage. Considering the results in all these experiments it appeared to make little difference at what time after spraying the plants were covered, if not more than a few days, provided the time spent in this saturated atmosphere was the same.

It was noted that under the bell jars the plants were dripping with moisture; not only was the air saturated, but the surfaces of the plants were covered with films and drops of water. To learn if an increased amount of humidity is of significance when kept below the point of saturation, sets of tomato plants (three in a pot), were sprayed and dried, then one set was put under a tightly closed bell jar, one was put under a bell jar with the stopper removed and with a tiny crack underneath, and a third was left outside. All were kept in strong diffused light.

Under the jar with the stopper removed were placed wet and dry bulb thermometers and a tiny fan run by a toy motor. This was used to fan the wet bulb for one minute before reading the temperature.

The procedure was to spray 9 tomato plants, three sets of 3 in a pot, with the same mixture, let them dry for a few minutes, then inclose one set tightly under a bell jar 8 by 16 inches on a ground glass plate, inclose another set under a bell jar slightly open at the top and bottom as described above, and keep the third set on the table beside them without covering. After standing under these conditions for three days the plants were all carried to the adjoining greenhouse, where they were left uncovered for about 10 days, after which notes were taken.



Six such experiments were carried out, of which the one shown in Table XVIII is representative. In this case the plants were sprayed with calcium arsenite<sup>14</sup> (1.95 gm., in 2 liters of water; no soap added).

It will be seen that the humidity does not need to reach the saturation point (100 per cent relative humidity) to materially increase the injury over that in a drier atmosphere, although in the saturated atmosphere still greater injury was done. Presumably, other conditions being equal, the degree of injury will be in a certain relation to the relative humidity.

Wetting the leaves at frequent intervals after spraying did not, however, have the same effect as a humid atmosphere.

TABLE XVIII.—Showing the increased injury caused by humidity produced by transpiration under bell jars

Conditions.	Time.	Temperatures.			Degree of injury.
		Wet bulb.	Dry bulb.	Relative humidity.	
		F.	F.		
Under bell jar closed....	(a)	.....	.....	100	Plant dead.
Under bell jar partly closed.	8 a. m..	58°	62°	79	} Partly defoliated.
	1 p. m..	64	66	90	
	5 p. m..	64	66	90	
	8 a. m..	60	62	89	
	1 p. m..	64	66	90	
	5 p. m..	64	66	90	
	8 a. m..	56	58	89	
	1 p. m..	58	60	89	
Not under bell jars.....	8 a. m..	57	64	65	} Injury moderate.
	1 p. m..	60	68	63	
	5 p. m..	61	70	61	
	8 a. m..	56	64	60	
	1 p. m..	58	68	54	
	5 p. m..	59	67	62	
	8 a. m..	53	60	32	
	1 p. m..	55	61	68	

\* Throughout.

Both in the greenhouse and out of doors sprayed foliage was given a light application of water through a fine spray nozzle every few hours throughout the day. The surrounding air was hot and dry and the leaves dried off very soon after each application. Under these conditions, if no soap had been mixed with the arsenical, the injury was not in any case noticeably greater than on the control plants that were sprayed with the same arsenical but not sprayed with water afterwards. In case soap had been added there was a slight tendency to increase the injury by subsequent sprayings with water, but this injury was incomparably less than produced by a humid atmosphere.

#### EFFECTS OF SOIL MOISTURE

As plants and trees can not always receive a sufficient amount of moisture through the roots it is but natural in spraying operations where foliage burning is liable to occur to raise the question as to whether or not a wilted condition of the leaves would affect the extent of injury.

<sup>14</sup> Baker's.

Greenhouse conditions have been found best for determining the answer, and we have made innumerable trials on tomatoes, beans, and other plants. When slightly or moderately wilted plants were sprayed in comparison with turgid ones, the resulting injury has been invariably practically the same. Any differences that have been noted were within the experimental error. The only cases in which there was a marked difference was when the wilting was so extreme and so long continued that the leaves were practically dying.

#### EFFECTS OF TEMPERATURE

A factor that might be supposed to affect arsenical injury is the temperature of the leaves and the surrounding air. In an effort to determine the effect of temperature upon the extent of injury to plants sprayed with arsenicals several series were run in a differential thermostat. This consisted of a series of chambers in which were maintained temperatures varying from 5° to 40° C. Light was admitted through glass at the top. Sprayed tomato and cucumber plants were placed in this thermostat for two days and then transferred to an open bench in the greenhouse.

The arsenicals used were diplumbic lead arsenate and Paris green.<sup>15</sup> It was found that between 5° and 15° C. there was little difference in the injury. From 15° to 25° the injury materially increased. Above 25° there was apparently a marked increase in the injury, but the unsprayed controls could not be kept in a healthy condition at these temperatures. Hence the records on the arsenical injury to sprayed plants at corresponding temperatures were of doubtful value.

These experiments show that an elevation of temperature increases the injury, but we wish to emphasize that under field and orchard conditions temperature is of minor importance as compared with humidity.

#### EFFECTS OF WOUNDING

In connection with studies made on the effects of arsenical compounds on the bark of fruit trees, we have shown (29, 30) that the all-important factor in determining whether or not injury will occur is the integrity of the outer corky layers of the bark, i. e., whether or not this has natural or artificial openings. Are wounds important in determining the extent of injury through the foliage? We have often noticed that there is an excessive injury at the margins of the wounds made by hail stones, whipping by the wind, and other mechanical agencies. In some instances we have purposely made such wounds before spraying. In some of these cases the injury was strictly confined to the margins of such wounds, but this was only where the arsenical treatment was mild. In some instances where the injury was confined to a few scattered spots there was evidence of a tiny puncture in each spot, revealed only by the aid of a lens. Wherever the arsenical treatment was so severe as to injure the foliage badly, there was every evidence of absorption directly through the unbroken epidermis. The injury at the margins of wounds is strictly local, usually making a brown strip about one-eighth of an inch wide and, except where such wounds were unusually extensive, this injury is of little practical importance.

<sup>15</sup> Both Baker's.

## EFFECTS OF SHAKING BRANCHES AFTER SPRAYING

Both in the practical orchard and field spraying and in experimental work there is often more or less wind that shakes some of the liquid from the leaves. It is important to know, therefore, the extent to which this will decrease the amount of arsenical injury to the foliage. In all the experimental work of this investigation the spraying was done when there was little or no wind, but this was probably an unnecessary precaution. On several occasions apple limbs were sprayed in duplicate, one being shaken vigorously immediately afterwards and the other hanging quietly until the spray mixture had dried on. Similar experiments were conducted with potted plants in the greenhouse. In no case was there a marked reduction in the amount of burning and usually no difference could be detected. As this shaking removed the excess liquid from the leaves so much more quickly and thoroughly than an ordinary wind, it is fair to conclude that any wind not strong enough to practically prevent spraying operations will have no material effect on the amount of arsenical injury.

## EFFECTS OF LIGHT AND DARKNESS

Three experiments were conducted to determine if light increases or decreases the amount of injury caused by arsenical spraying.

Potted tomato plants were placed in a dark room in the evening. Early the next morning two were sprayed with calcium arsenite and put under bell jars in dark boxes wrapped with black cloth; two were sprayed and put under bell jars not in dark boxes, and unsprayed controls were kept under both these conditions. All boxes and bell jars were kept in a strong diffused light for two days and then the plants were removed and placed on a bench in the greenhouse. In two of the experiments the injury to the sprayed plants was equally bad in the dark boxes and in the light. In the third, all the sprayed plants were killed, making comparisons impossible. The unsprayed plants remained in healthy condition.

## SIGNIFICANCE OF THE RESULTS

The place of arsenical compounds in the category of insecticides is so important that we can not at present expect their replacement by any other group of chemicals. They have their faults, however, and among these is the marked tendency to injury of the crop they are intended to protect. It is therefore of prime importance to learn the factors that determine this injury and with this knowledge to reduce it to a minimum. Some of the existing beliefs regarding this injury are founded on fact, the difficulty being to know the extent of the tendency. Other beliefs appear to be based on a wrong conception.

It has been found quite difficult to make exact statements concerning the toxicity of the different arsenicals because of the variability of their composition. This is especially true of the lead arsenates. Theoretically one might expect ortho, meta and pyro arsenates each in the form of monoplumbic, diplumbic, and triplumbic salts, a total of 9 possibilities. Actually the number that form is fewer, but, on the other hand, many commercial lead arsenates are not single salts of lead and an arsenic acid but a mixture of different lead arsenates. Furthermore, the product of a company may change from time to time, and even within a short

period different lots from the same factory may vary. It is encouraging that the product of certain companies is becoming stabilized, and it is to be hoped that others will follow their example. At the present time, however, we can not make definite statements about lead arsenate without specifying the brand and lot. These remarks apply in some degree to zinc arsenite and calcium arsenite, while Paris green and arsenic trioxid are much more uniform in composition.

It is rather surprising that calcium arsenate should ever have been used as extensively as it was some 20 years ago when one observes its tendency to burn foliage. To be sure, the competition with other arsenicals, e. g., lead arsenate, did not then exist, and in the less frequent injury by these compounds we find one of the chief causes of its progressive abandonment.

The most striking example of a poorly founded prejudice is the quite general belief that arsenic trioxid (common white arsenic) will burn foliage even when occurring as a very small impurity in other arsenicals. We are not able to explain fully this belief. Probably it lies partly in the fact that chemists expressed their findings of soluble arsenic in insecticides in terms of "arsenious acid," the reasons for which are not always clear to others. Probably also, the letting of the spray mixture stand for hours or days before applying increases the danger from arsenic trioxid more than from other arsenical compounds, and has been a factor. In any event, we feel that we have established quite conclusively that a freshly prepared suspension of arsenic trioxid is not particularly harmful to foliage, and we entertain a hope that this relatively cheap form of arsenic may come into general use for the control of certain insects. Of course its efficacy as an insecticide has yet to be determined.

The hope expressed by Gillette (11) that arsenic trisulphid would prove less injurious than the arsenical insecticides in common use, seems unlikely of realization, for, while this compound has a low solubility in pure water, it nevertheless causes considerable burning when sprayed upon foliage.

In the selection of an arsenical insecticide for any crop to be sprayed, the entomologist will do well to consult the table of susceptibility on page 512, for on some plants a mixture may be used that would be quite destructive to others.

The practice of adding to an arsenical spray mixture some other material to act as a supplementary insecticide or fungicide, or as a "sticker" or "spreader," or to decrease the tendency to injury, has been the subject of considerable controversy. Conspicuous among these materials is soap, which, in addition to being a spreader, has been shown by Parker (22) to be of considerable value in keeping lead arsenate in suspension. That soap increases the tendency to burning by nearly all "insoluble" arsenicals is clearly established (p. 523), but it is safe to say that the increase in burning tendency with lead arsenate is not sufficient to discourage its use on the more resistant plants such as cabbage, sugar beets, potatoes, and apple. It is indeed a fortunate circumstance that cabbage and sugar beets, on which a spreader is a necessity, are resistant enough to arsenical injury to make the use of soap permissible. It is likewise fortunate that soap has been found to actually decrease the injurious properties of Paris green, for gardeners may now rest assured that the addition of soap to it is not only permissible but desirable. Lime sulphur increases the injury by nearly all the arsenical insecticides in about the same degree that soap does, and must not be used in com-

bination with them on very tender foliage. The fact that lime sulphur decreases the injury caused by calcium arsenite appears to be of only scientific interest, for even with this addition the burning will be so severe that this arsenical is not useable under most circumstances. Tobacco extract gives no increase in the injury by any of the arsenicals.

Of the environmental conditions, only humidity seems to be of large significance, for while the burning is increased in a measure by a rise in temperature, it is doubtful if this tendency is great enough within the range of temperatures encountered in the field to be entitled to practical consideration. The effect of humidity, however, can hardly be overrated and must be given large consideration in all spraying operations. To be sure, this factor is not under the control of the orchardist and farmer, but he can make a fair estimate of the humidity that is likely to prevail following spraying in his locality, and be bold or cautious in the selection of his spraying material.

The varying results observed in this investigation when spraying experiments were repeated under conditions identical, except as to weather, show the significance of atmospheric conditions, but, what is of especial importance to the investigator, these varying results show the great danger in drawing conclusions from single experiments.

#### SUMMARY

Based on an investigation extending through a period of 10 years, and involving the application of various arsenical spray mixtures to approximately 10,000 separate plants and branches of trees sprayed individually, we submit the following conclusions concerning arsenical injury to foliage:

(1) The name commonly used does not indicate the composition of an arsenical with sufficient exactness. This is especially true of the calcium arsenites, lead arsenates, and zinc arsenites, in which the results obtained by using different lots labeled the same except as to manufacturer may give widely divergent results. The arsenic trioxids and Paris greens are much more uniform in composition.

(2) The arsenical insecticides least injurious to foliage are iron arsenate and certain of the lead arsenates. Possibly new ones will be proposed that will be as safe or safer.

(3) Of the lead arsenates not all pure diplumbic ortho-lead arsenates are identical in burning properties, nor are all triplumbic ortho-lead arsenates identical in this respect. Some diplumbic lead arsenates are as safe to use as some of the triplumbic ones.

(4) Arsenic trioxid is not so dangerous to the foliage as is generally supposed, and indeed this compound is permissible as an insecticide on any but the most delicate foliage, provided it is applied promptly after mixing with water.

(5) Standing after mixing causes a very marked increase in injury by arsenic trioxid and a slight increase in injury by other arsenical insecticides.

(6) Of the readily soluble arsenical compounds cacodylic acid and sodium and potassium cacodylates proved the most injurious. This is quite in contrast to the well recognized fact that these compounds of arsenic are less harmful to the higher animals than most others.

(7) The foliage is more susceptible to arsenical injury than the fruit or the stems.

(8) The injury to leaves is characterized, first, by a lack of luster, then wilting, and a final change to some shade of brown (dependent upon the species of plant) as the affected tissue becomes dead and brittle. The symptoms are not sufficiently distinctive to separate arsenical injury from some others.

(9) The injury to the foliage is practically all through the lower epidermis, regardless of the numbers of stomata in the two surfaces, indicating that it is a result of direct penetration of the thinner cuticle.

(10) Individual plants of the same species and variety vary somewhat in their susceptibility to arsenical injury.

(11) The older leaves of a plant are more susceptible than the younger ones.

(12) Soap added to soluble arsenicals offers a slight protective action.

(13) Soap added to most insoluble arsenicals increases the injury by increasing the solubility to a point more than counteracting its slight protective action.

(14) Soap added to Paris green in suspension distinctly restrains the burning of foliage.

(15) Gelatin, milk, and agar do not increase the arsenical injury to foliage.

(16) Lime sulphur increases the injury caused by most insoluble arsenical compounds.

(17) Lime sulphur distinctly decreases the injury caused by calcium arsenite but not to a sufficient extent to make this a safe insecticide.

(18) Tobacco extract has little influence upon the injurious properties of arsenical insecticides.

(19) Lime restrains, to some extent, the injury by calcium arsenite and Paris green.

(20) We have not been able to decrease the zinc arsenite injury by adding ferrous sulphid.

(21) Repeated spraying with zinc arsenite is liable to result in serious burning.

(22) A slightly wilted condition of the foliage does not result in increased injury.

(23) Light seems not to be an important factor in arsenical injury to foliage.

(24) An increase in atmospheric temperature results in a moderate increase in arsenical injury; but within the ranges of temperature found during the summer in a suitable orchard climate this is of little practical importance if the air is relatively dry.

(25) Humidity is the greatest environmental factor in determining arsenical injury to foliage, and this influence is very marked even before the saturation point is reached.

(26) Using a few experiments as a basis for generalization upon arsenical injury is a treacherous proceeding and may lead to erroneous conclusions. The only safe procedure is to test repeatedly each point under consideration.

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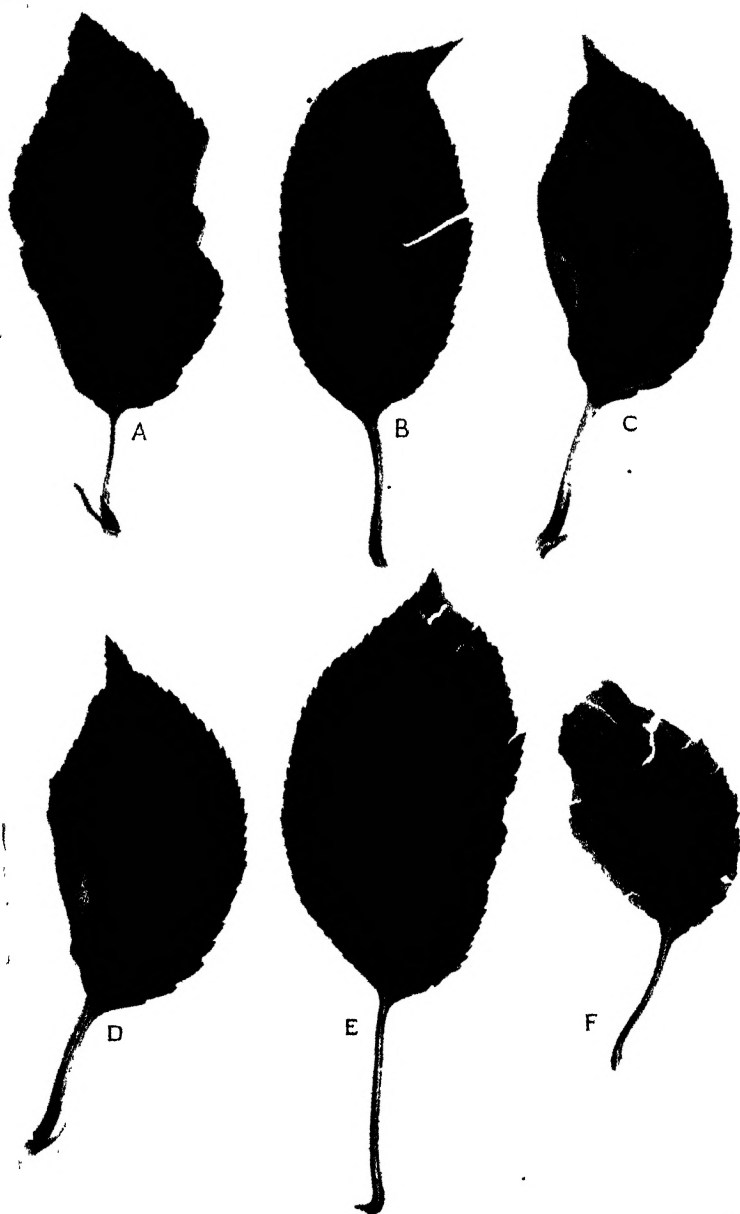
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PLATE I

■ Stages in the development of arsenical spray injury on apple foliage.

- A.—Three days after spraying.
- B.—Four days after spraying.
- C.—Five days after spraying.
- D.—Seven days after spraying.
- E.—Ten days after spraying.
- F.—Twenty-five days after spraying.





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